Canadian Journal
of Research

Issued by THE NATIONAL RESEARCH COUNCIL of CANADA

VOLUME 5 JULY, 1931

STUDIES ON LIGNIN AND RELATED COMPOUNDS. VIII. A KINETIC STUDY OF THE ACTION OF SULPHUROUS ACID ON LIGNIN AND RELATED COMPOUNDS[1]

BY CHARLES A. SANKEY[2] AND HAROLD HIBBERT[3]

Abstract

Electrical conductivity measurements have been made over the temperature range 18-145°C. of reaction mixtures of sulphurous acid and a large number of type compounds, and data obtained on the stability of the resulting sulphonic acids and the mobility of the equilibria involved. The compounds investigated include saturated and unsaturated aldehydes; saturated, unsaturated and cyclic ketones; phenols; substances in which an ethylene linkage is the only reactive group; furane derivatives; and substances containing pyrone rings.

The results are discussed from the standpoints of structure and reaction mechanism. The sulphonic acids of nuclear aldehydes, saturated ketones and cylic ketones are typically unstable. With compounds containing an ethylene linkage conjugated with a carbonyl group, addition normally takes place in the 1:4 positions and is followed by hydrogen migration. Where an ethylene linkage is the only reactive group present, addition proceeds only very slowly and at high temperatures. The difficulty of effecting a reaction between sulphurous acid and phenols in their tautomeric alicyclic forms is indicated by the non-reactivity of resorcinol, and the formation of only a small percentage of an extremely unstable derivative by phloroglucinol. Furfuryl alcohol and glucal are shown to form oxonium derivatives which serve as intermediates for the entry of the sulphonic acid group into the ring system.

Conductivity-temperature curves for lignosulphonic acids from various sources indicate that the products are sulphonic acids of the $-C=C-$type. Other evidence points to an oxonium addition product with a ring oxygen as being a probable intermediate in the formation of the final and more stable lignosulphonic acid. The relation of such a mechanism to the experimental conditions employed in the commercial process of the manufacture of sulphite pulp is indicated.

The formation of lignosulphonic acids through tautomerization of a phenolic nucleus is highly improbable. Also for such formation a carbonyl group does not necessarily have to be present. Strong additional evidence is presented for the presence of a heterocyclic ring containing oxygen and for an ethylene linkage in the lignin molecule. The latter is indicated to be in the same ring system as the oxygen atom.

Manuscript received February 10, 1931.

Contribution from the Industrial and Cellulose Chemistry Laboratories, McGill University, Montreal, Canada, with financial assistance from the Canadian Pulp and Paper Association. Constructed from a thesis submitted to the Faculty of Graduate Studies and Research of McGill University by Charles A. Sankey, in May, 1930, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The first part of this study appeared in the Journal of Physical Chemistry (27).

[2] *Postgraduate student at McGill University and holder of a studentship under the National Research Council of Canada.*

[3] *Professor of Industrial and Cellulose Chemistry, McGill University.*

Introduction

The necessity for a kinetic as opposed to a purely organic chemical approach to the lignin problem has already been emphasized in these communications (25, 26, 27). The present investigation, which was commenced by Hoover and Hunten (27), had as its first object a kinetic study of the products of reaction of sulphurous acid with substances of known constitution containing chemical groupings similar to those which have been indicated as present in lignin, and as its second an extension of this study to the lignosulphonic acids.

The active part of the lignin complex in its reaction with sulphurous acid may be (a) an actual or potential carbonyl group; (b) an actual or potential ethylene linkage; (c) a heterocyclic ring containing an oxygen atom or atoms capable of oxonium salt formation; or (d) any combination of the above.

Evidence for the Presence of Various Groups in Lignin

Carbonyl Group

The presence of a carbonyl group has been indicated by the isolation by Friedrich and Diwald (7) of a condensation product of a specially prepared lignin with phenylhydrazine; by the further investigations of Friedrich (6), who claims to have established the existence of a tautomeric —CO—CH_2— group by isolation of two soluble forms of lignin which are interconvertible; and by the assumed formation of lignin acetals by Hägglund and Urban (20), who extracted wood meal with isoamyl alcohol using hydrochloric acid as a catalyst. Various polyalcohols have also been employed in this laboratory for the isolation of lignin (22, 23, 26), but there is some doubt as to whether the products are lignin acetals. The presence of active carbonyl groups has been assumed in the "lignin formulas" of Cross and Bevan (3), Schrauth (48), and Klason (33).

The majority of present workers however prefer to consider the carbonyl group as latent. Thus Fuchs (10) suggested that it may be formed by ketonization of a phenolic nucleus, while Klason in putting forward his coniferyl paraldehyde formula for lignin (34, 35) indicated a type of latency due to polymerization. Hägglund, Johnson and Trygg (19) postulated a similar reason for the assumed latency. Another possibility is that a carbonyl group is formed by loss of water from the molecule, followed by a keto-enol shift, as suggested by Freudenberg, Zocher and Dürr (5). A latent carbonyl group is also present in flavones and flavonols (18). The evidence for this latter type of structure will be discussed later.

Ethylene Linkage

Klason has assumed that in lignosulphonic acids the sulphonic acid group adds on directly to an ethylene linkage and that an aldehyde group is also present. This idea (30, 32, 33, 34, 35), culminating in the coniferyl paraldehyde formula already mentioned, is based on the presence of the glucoside coniferin in the cambial sap, and on his experiments with β-naphthylamine derivatives of lignosulphonic acids. The developments from the latter are practically pure speculation made in order to account for his analytical results, which are

not at all convincing. As Pauly and Feuerstein (43) have shown, some of Klason's experimental work is in serious error.

Better evidence is given by Fuchs (13), who claims that a glucal complex is present in lignin. More recently Fuchs and Horn (16) have shown that the action of bromine dissolved in carbon tetrachloride on an acetylated pine wood is analogous to that on a partially hydrogenated benzene nucleus. They claim that their data exclude the possibility of a larger ring, or a straight chain being involved, and therefore assume lignin to contain a tetrahydrobenzene ring. Reference has already been made to Friedrich's —CO—CH_2— tautomers (6).

On the other hand Rassow and Zickmann (46) state that lignin is unaltered by reduction with aluminium or zinc and acetic acid and is therefore saturated. It has been shown in Part V of this series (25) that dry chlorine has no action on dry spruce meal. This non-reactivity with halogens must be somewhat discounted however, since unsaturated compounds are known which will not react with bromine (50).

Ring Oxygen Atom Capable of Oxonium Salt Formation

The formation of oxonium derivatives by pyrones has long been established while a similar possibility for furane oxygen has recently been pointed out by Moureu, Dufraisse and Johnson (41). Those who consider lignin as being derived from the condensation of 2, 5-anhydroglucose units assume it to contain several five-membered heterocyclic rings containing oxygen. The formulas of Schrauth (48) and of Jonas (28) are derived on this basis. These rings have not a true furane character since they are saturated. The formula of Jonas does however contain an ethylene linkage. Under drastic experimental conditions 5-hydroxy-methylfurfural has been obtained from lignin (11, 12).

Rassow and Zickmann (46) insist on the presence of a pyrone ring stating that lignin contains the essential skeleton of a 2-phenyl flavone. Using Willstätter lignin, they claim that the removal of the dark color of the product on washing with water is due to the decomposition of an oxonium salt. A recent paper by Fuchs and Horn (17) also supports this view. They report that both HCl-lignin and acetyl-lignin react with glycol methyl ether in the presence of hydrochloric acid to yield a product containing three more free hydroxyl groups than were present in the original substance. Two of these hydroxyl groups are capable of acetal formation and the third is phenolic. The reaction is therefore claimed to be the transformation of a mixed aromatic heterocyclic derivative into a keto-hydroxy body. The heterocyclic grouping is also claimed to be present in genuine lignin but is much more readily subject to chemical action in this original form than in the isolated product, *e.g.*, it is much more readily acetylated.

The flavone type of structure would also account very well for combination between the carbohydrates of the cell wall and lignin (analogously to the anthocyanins), such as the ether or ester linkage between the polysaccharides and lignin postulated by Friese (8) or the glucal complex of Fuchs (13, 14), and would explain the almost limitless tendency of lignin to give color reactions.

The suggestion is also reasonable in view of the wide distribution of such products in plant life. On the other hand lignin can hardly be a simple flavone. These compounds yield well-defined hydrolysis products which indicate their constitution.

Previous Investigations

The first part of the present research, begun by Hoover, Hunten and Sankey (27), comprised measurements of the interaction products of sulphurous acid and certain aldehydes over the range of temperature normally used in the process of sulphite cooking, *viz.* 18-145° C. Provided that the data so obtained could be interpreted as a constitutive property of the original aldehyde, the investigation was to be extended to other compounds of known constitution and finally to the lignosulphonic acids themselves.

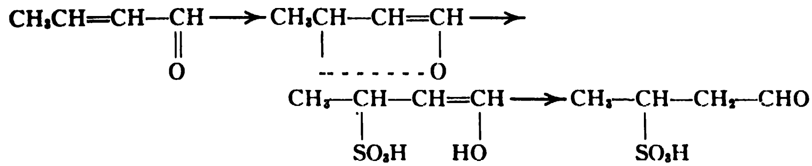
Electrical conductivity studies of lignosulphonic acids have been made previously by Melander (40) who titrated certain lignosulphonic acid fractions with sodium hydroxide and determined the equivalent weight by conductivity measurement, the conductivity passing through a minimum at the equivalent point. He also reported that the lignosulphonates were quite highly dissociated. Conductivity measurements were also made by König (37), who found that the acids were strong acids whose sodium salts were not hydrolyzed. Finally Kerp (29) studied the conductivity of formaldehyde sulphonic acid, and also of acetaldehyde sulphonic acid over a more limited range of conditions, and calculated the degree of dissociation for both. The figures for these, and also for the change of equivalent conductivity with dilution, presented fairly conclusive evidence that the compounds were strong acids.

Hoover, Hunten and Sankey (27) investigated the sulphonic acids of butyraldehyde, crotonaldehyde, cinnamic aldehyde and hydrocinnamic aldehyde. The apparatus described by them for making and handling the compounds in an atmosphere of nitrogen was used throughout the present investigation. The conductivity cell was a modification of Campbell's (2) instrument.

Measurements of specific conductivity were made for these systems over the temperature range 18-145° C. Two types of curves were obtained. (1) *From saturated aldehydes:* In these cases the conductivity rose with increasing temperature to a maximum at about 65-75° C. and then decreased up to the highest temperatures at which measurements were made. (2) *From unsaturated aldehydes:* With these, on first heating the reaction mixture in the conductivity cell, the specific conductivity followed the same course as for saturated aldehydes, but after passing through the usual maximum broke away and rose steeply at a temperature of 100-110° C., thereafter continuing to rise with increasing temperature. The path of the downward curve (taking readings with decreasing temperature) was not identical with the upward but showed no "breaks" and the conductivity fell continuously. On heating the cell a second time the downward curve was repeated. The character of the curve was taken as showing the presence of a sulphonic acid involving the ethylene linkage, *e.g.*, (I) or (II) from crotonaldehyde.

(I) $CH_3CH(SO_3H)CH_2CHO$ (II) $CH_3CH_2CH(SO_3H)CHO$

It should be noted that if the reaction involved essentially a 1:4 addition followed by hydrogen migration then (I) would be formed thus



while if the action was one of direct addition to the ethylene linkage both (I) and (II) would result.

It was further shown that at room temperature even in the presence of excess sulphurous acid, addition took place to the aldehyde group only, yielding $CH_3CH=CH—CH(OH)SO_3H$.

The reaction products were proved to be strong acids by their $\Lambda - \log D$ curves and therefore sulphonic acids ($R—CH(OH)—SO_3H$), and not sulphite esters ($R—CH(OH)—O—SO_2H$). This confirmed the conclusions of Stelling (49) based on X-ray analysis.

Systems Studied

Nuclear Aldehydes

The reaction of aldehydes in which the $-CHO$ group is connected directly to the benzene nucleus is of interest since Freudenberg, Harder and Markert (4) hold that a nucleus of the vanillin type is present in lignin, and Kürschner (38) claims to have obtained large yields of vanillic acid from Willstätter lignin by sublimation at 190-210° C. Two such aldehydes were therefore studied, namely, benzaldehyde and vanillin.

System:— Benzaldehyde—Sulphurous Acid

With this system, while a sulphonic acid is readily formed, it is much less stable than any of the other aldehyde sulphonic acids previously studied. Thus in approximately 0.1 *M* solution, for an equimolar reaction mixture, the equilibrium was about 92% on the side of the sulphonic acid at 20° C. but it is so readily shifted that when free sulphurous acid was determined by the usual iodometric method high results were obtained. To get accurate figures it was found necessary to freeze the equilibrium by running the sample into a very slight excess of iodine in a beaker containing ice and completing the titration as rapidly as possible.

The equilibrium is also shifted towards the left on dilution. Thus if a 0.1 *M* solution be diluted ten times, the sulphonic acid in the resulting solution will be less than 0.01 *M*. A comparison of the calculated dilution (0.1 $M=$ 100 litres) with the true dilution (analysis = 116 litres) obviously measures the equilibrium shift. The values obtained for this sulphonic acid are shown in Fig. 1, curve *C*. The other curves in this figure indicate similar data for other sulphonic acids studied. A greater slope of the curve corresponds to increased decomposition of the sulphonic acid.

The ease of displacement of the equilibrium is also shown by the extreme

readiness with which the sulphonic acid is hydrolyzed by barium hydroxide. In the basic medium of excess of this base, free sulphurous acid is precipitated

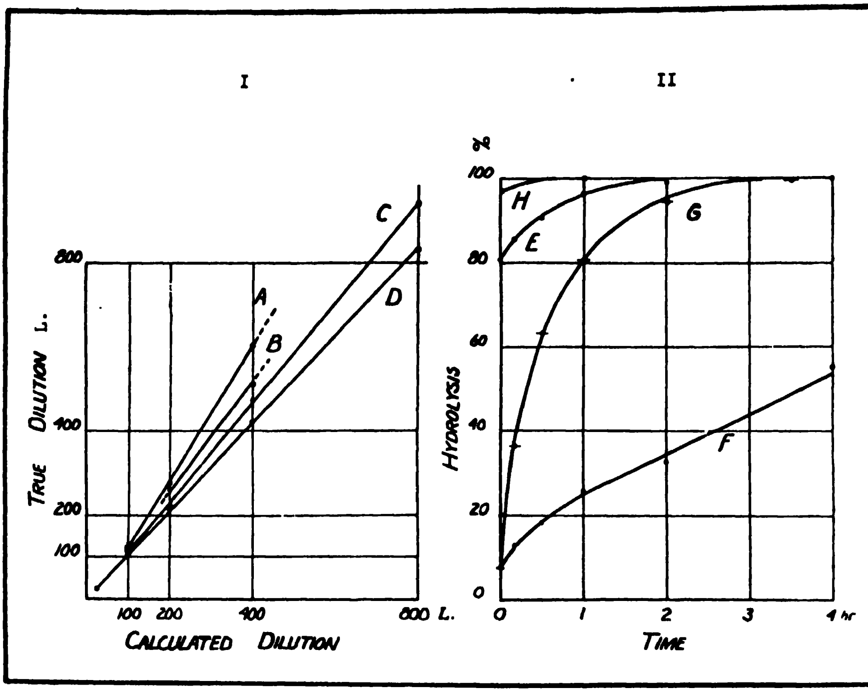


FIG. 1. *Effect of dilution on the equilibrium:* $R.CO.R'+H_2SO_3 \rightleftharpoons R.C(OH)SO_3H.R'$. *A. Methyl ethyl ketone* (5 × *excess*). *B. Vanillin* (*equimolar*). *C. Benzaldehyde* (*equimolar*). *D. Cyclohexanone* (2.5 × *excess*). *II. Hydrolysis of sulphonic acids with barium hydroxide. E. —CHO acid of crotonaldehyde* (0.0329 *M*). *F. —C=C— acid of crotonaldehyde* (0.0336 *M*). *G. Benzylidene acetone sulphonic acid* (0.0328 *M*). *H. Benzaldehyde sulphonic acid* (0.1032 *M*).

as barium sulphite and the equilibrium thus shifted. Provided that the reaction takes place in a nitrogen atmosphere to prevent the oxidation of sulphite to sulphate, the total sulphite may be readily determined at the end of a given time by acidifying the mixture and analyzing iodometrically, and the course of the hydrolysis thus followed. This method was used extensively later, as a measure of the stability of sulphonic acids. With benzaldehyde sulphonic acid, hydrolysis is over 95% complete almost immediately and 100% complete in about 10 min., a very rapid rate as shown by curve *H* of Fig. 1.

Conductivity measurements were made over the usual range of temperature at 229 litres dilution (sulphonic acid). The curve obtained is similar to that for vanillin as shown in Fig. 2. The type is that of the usual saturated aldehyde except that the down curve breaks slightly away from that obtained with rising temperature and indicates by its higher maximum an increased concentration of sulphurous acid. There is therefore at higher temperatures an apparently lasting decomposition of the sulphonic acid, in other words recombination does not occur to a sufficient extent to restore the

former equilibrium. A second conductivity run was made at the same dilution, with 110° C. as the maximum temperature. A similar type of curve was obtained showing to a somewhat lesser extent the sulphonic acid decomposition noted above.

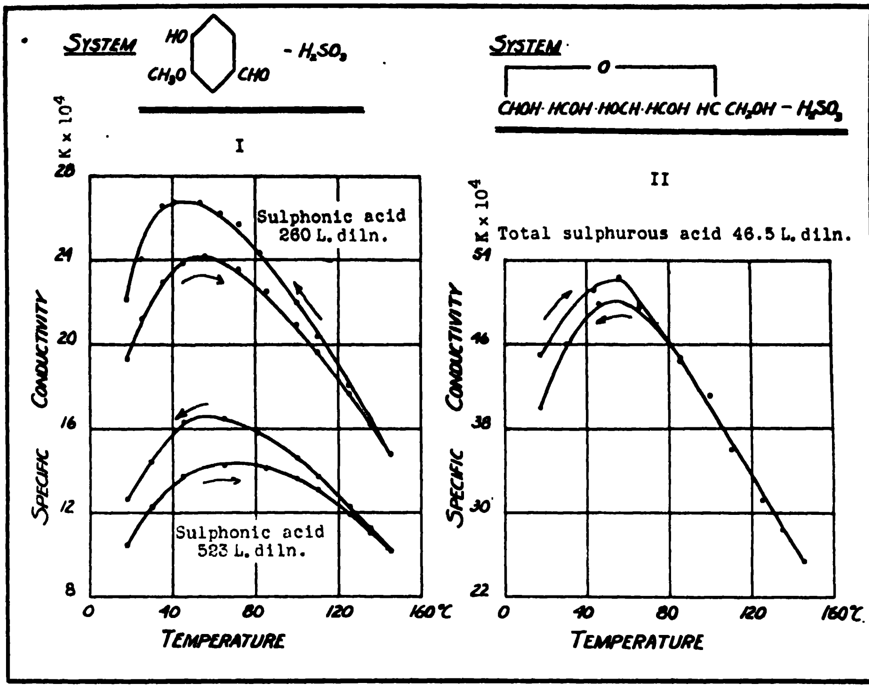


FIG. 2. *I. Specific conductivity of vanillin-sulphurous acid system.*
II. Specific conductivity of glucose-sulphurous acid system.

System:— Vanillin—Sulphurous Acid

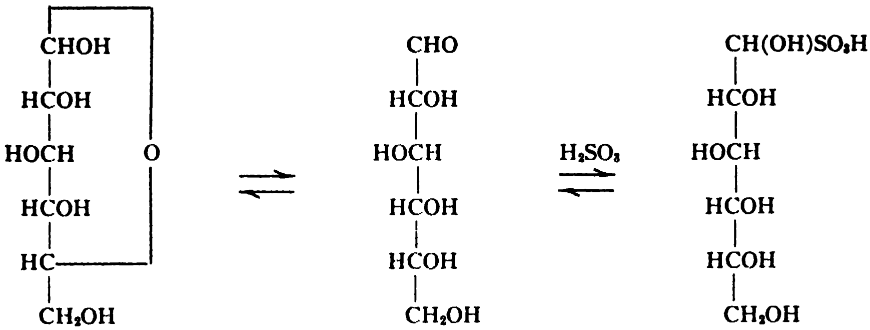
The characteristics of this system were found to be similar to those noted for the benzaldehyde reaction mixtures except that the sulphonic acid was even more unstable, probably due to the influence of the *p*-hydroxyl in the benzene ring. Thus curve *B*, Fig. 1, shows the equilibrium shift on dilution and, by its greater slope, indicates greater instability than in the case of benzaldehyde sulphonic acid. The rate of baryta hydrolysis is as rapid as that of the system previously discussed. The latter method is, of course, less sensitive for determining differences between acids that are easily hydrolyzable than between more stable ones.

Conductivity-temperature data were obtained at sulphonic acid dilutions of 260 and 523 litres over the 18-145° C. range (Fig. 2). As in the case of benzaldehyde one run was made with 110° C. as the maximum temperature. The curves showed the same high temperature decomposition of sulphonic acid.

System:— Glucose—Sulphurous Acid

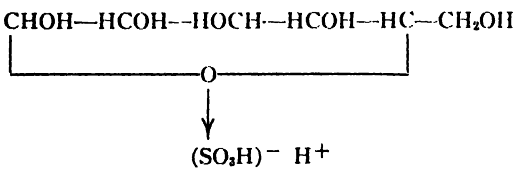
This system was selected in view of the essentially latent character of the

aldehyde group in glucose, since in solution the free aldehyde concentration is to be regarded as very low. The probable reactions involved are those shown in the scheme:



Analysis showed the reaction proceeded slowly. In equimolar solution (0.0770 *M*) only 6.5% of the total sulphurous acid combined in five days. The sulphonic acid percentage was raised to 14.9% by removing excess sulphur dioxide in vacuum. The conductivity-temperature curve, Fig. 2, indicates that the sulphonic acid is probably nearly all decomposed at temperatures over 60° C.

A possible alternative to the above mechanism was suggested by later experiments with furfuryl alcohol and with glucal (*q.v.*), namely, that the reaction product was an oxonium derivative (IIa). In the lack of other evidence however that glucose is capable of forming such compounds, the authors prefer to believe that the very slow realization of equilibrium concentration of the sulphonic acid is indicative of a mechanism of formation through an intermediate whose concentration in the reaction mixture is always low. This fact is of considerable interest as bearing on the cyclic-open chain glucose equilibrium in solution. It would seem to offer confirmatory evidence as to the probable presence of the free aldehyde form in only relatively small amount.



(IIa)

KETONES

The interaction of sulphurous acid with various types of ketones has also been investigated. The ketones studied were methyl ethyl ketone, benzylidene acetone, dibenzyl ketone and dibenzoyl methane.

System:— Methyl Ethyl Ketone—Sulphurous Acid

A study of the interaction of this typical saturated ketone and sulphurous acid emphasizes the characteristic difference between aldehyde- and ketone-sulphonic acids. The latter are much more unstable. The equilibrium is usually much further to the left than in the case of the corresponding aldehyde equilibrium and is also much more readily displaced. This is shown by the

equilibrium shift on dilution (Fig. 1, curve *A*). The curve is from data on a system containing five times the theoretical amount of ketone so as to force the equilibrium to the right in order to get conductivity curves more nearly approximating those of the sulphonic acid.

$$CH_3COCH_2CH_3 + H_2SO_3 \leftrightarrows \underset{\displaystyle SO_3H}{CH_3C(OH)CH_2CH_3}$$

Conductivity-temperature curves were obtained at sulphonic acid dilutions of 120, 278 and 603 litres, each with five times excess ketone. Curves for the last two dilutions are shown in Fig. 3. The curves are of the butyraldehyde

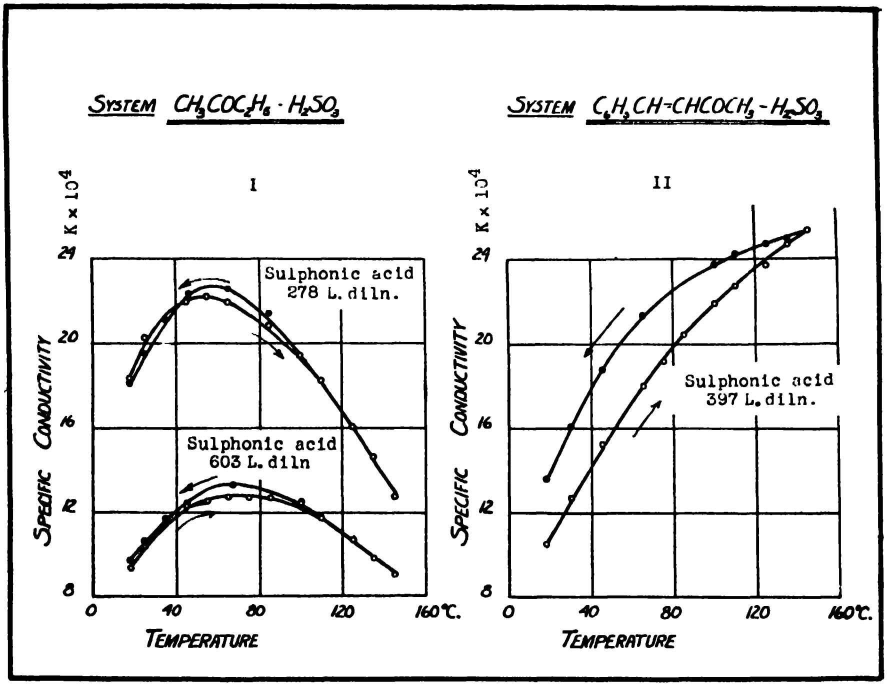


FIG. 3. *I. Specific conductivity of methyl ethyl ketone-sulphurous acid system.*
II. Specific conductivity of benzylidene acetone-sulphurous acid system.

type except that the maximum occurs at a lower temperature indicating earlier decomposition and that the down curves are more typical of sulphurous acid, showing that the recombination does not take place completely to give the former equilibrium on cooling the cell contents.

System:— Benzylidene Acetone—Sulphurous Acid

With this system equilibrium was only very slowly reached. In decimolar solution at 20° C. only 34% of the total sulphurous acid was combined after two days. The final equilibrium could however be approached by removing the excess sulphur dioxide in vacuum. Mixtures up to 83.7% sulphonic acid were obtained in this way without decomposition of sulphonic acid.

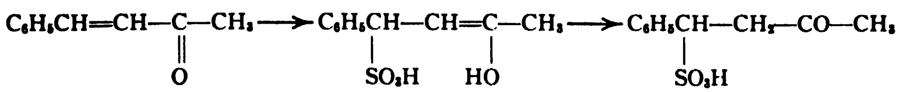
Conductivity-temperature curves were determined at sulphonic acid dilutions of 157 and 397 litres and showed a considerable and continuous rise with

increasing temperature over the whole range studied. The curve for the latter dilution is given in Fig. 3. The acid is therefore of the —C=C— type previously obtained for crotonaldehyde, *i.e.*, $C_6H_5CH(SO_3H)CH_2$—CO—CH_3.

If the sulphonic acid group were attached, initially, to the carbonyl group of the ketone, and were subsequently to change over at any temperature below the maximum of the usual curve for ketone sulphonic acids, *e.g.*, methyl ethyl ketone sulphonic acid, the fact would not be determinable from conductivity measurements alone. There is however considerable other evidence that the acid formed at 20° C. is much more stable than the sulphonic acid of a typical saturated ketone. In the first place the equilibrium of the latter is a very mobile one and is readily shifted on dilution and during iodometric determination of free sulphurous acid at room temperature. This is not the case with the sulphonic acid under discussion. Secondly, at the molarity of sulphonic acid corresponding to that obtained here the equilibrium is much further on the side of the sulphonic acid than for the corresponding compounds with saturated ketones (this acid, more than 83%; cyclohexanone sulphonic acid, approximately 60%; methyl ethyl ketone sulphonic acid, approximately 52%). The rate of hydrolysis for —C=C— acids is much slower than for —CHO acids. Typical curves for each of the crotonaldehyde sulphonic acids were obtained, as well as for saturated aldehyde sulphonic acids (benzaldehyde and vanillin), and these data, together with the observation of Hoover and Hunten that butyraldehyde sulphonic acid can be analytically determined from the sulphite produced by thirty minutes hydrolysis, are all in accord with this typical difference. The hydrolysis curves *E*, *F* and *G*, of Fig. 1, are for the acids $CH_3CH=CHCH(OH)SO_3H$; $CH_3CH(SO_3H)CH_2CHO$ and benzylidene acetone sulphonic acid respectively, each representing approximately the same sulphonic acid molarity. Curve *G* is of the ethylene linkage type. Since the acid in question is a ketone sulphonic acid it is of course more unstable than the corresponding crotonaldehyde compound.

The mechanism of this hydrolysis has been definitely established as involving a shift in the equilibrium through removal of free sulphurous acid as barium sulphite, since the results obtained by repeating the hydrolysis experiments using sodium hydroxide instead of barium hydroxide at the same normality showed no hydrolysis at 20° C. for reaction times up to four hours. Hoover and Hunten (27) had also previously observed that on attempting to hydrolyze butyraldehyde sulphonic acid with sodium hydroxide an equilibrium was set up for each concentration of the base.

It is not to be expected however that the actual mechanism consists of a direct addition to the double bond. As will be indicated later in the discussion of the reaction of sulphurous acid with certain unsaturated compounds, in which the ethylene linkage is the only active group, such direct addition takes place only slowly and at high temperatures. The present reaction proceeded at room temperature and hence it appears very probable that the addition takes place in the 1:4 position of the conjugated system followed by hydrogen migration according to the scheme:



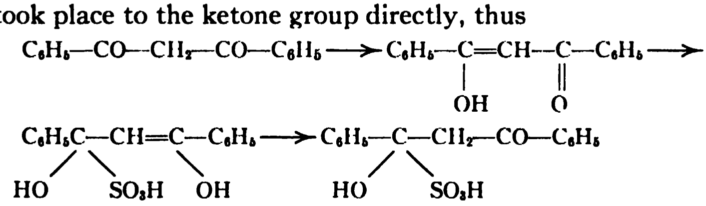
The fact that the equilibrium was attained slowly is in favor of this. Part of the delay in the establishment of the final conditions may have been due to the solid and insoluble character of the ketone, but the reaction mixture was kept well agitated and therefore, as in the case of glucose, the mechanism of the reaction would appear to involve the formation of an intermediate compound such as that postulated above, and whose concentration in the reaction mixture was always low.

System:— Dibenzyl Ketone—Sulphurous Acid

This ketone contains strongly negative groups attached to $-CH_2-CO-$ groupings and therefore should exist to a considerable extent in the enolic form. It does not react to form a sulphonic acid. Thus on analysis the figures for free sulphurous acid (iodine titration) and total sulphurous acid (sodium hydroxide titration) gave the same value. The amount of sulphur in the aqueous and non-aqueous (ketone) layers was ascertained by micro-analytical determination. The figure for the aqueous layer corresponded within experimental error with that obtained volumetrically for free sulphurous acid, while in the non-aqueous layer, a trace of sulphur present was easily attributable to slight mixing with the aqueous layer. Further no reaction took place when the mixture was heated to 145° C. for two hours. The non-reactivity within this range of conditions is therefore definitely established.

System:— Dibenzoyl Methane—Sulphurous Acid

Dibenzoyl methane exhibits keto-enol tautomerism with either carbonyl group. It does not react with sulphurous acid. It might be expected to behave similarly to dibenzyl ketone, for, assuming enolization of one of the carbonyl groups, followed by 1:4 addition to the resulting conjugated system and hydrogen migration, there would result exactly the same compound as if addition took place to the ketone group directly, thus



The non-reactivity therefore indicates that 1:2 addition does not take place and further confirms the influence of negative groups attached to a $-CH_2-CO-$ system.

Compounds Containing Cyclic Carbonyl Groups

In view of the possibility of formation of a carbonyl group by ketonization of a phenol nucleus in lignin, it was considered advisable to study the interaction of compounds containing cyclic carbonyl groups with sulphurous acid. Four such compounds were investigated, ranging from a cyclic ketone to certain phenols, namely, cyclohexanone, quinone, resorcinol and phloroglucinol.

System:— Cyclohexanone—Sulphurous Acid

The sulphonic acid of cyclohexanone, although still retaining the instability typical of ketone sulphonic acids, shows this property to a considerably smaller degree. Thus the equilibrium is further over on the side of the sulphonic acid than with methyl ethyl ketone and can be shifted to 95% sulphonic acid by use of two and one-half moles of ketone per mole of sulphurous acid. The equilibrium shift on dilution for the latter mixture is shown in Fig. 1, curve *D*. The greater stability over that of methyl ethyl ketone (curve *A*) at five times excess ketone is amply demonstrated. Due to the presence of this excess, the data are not directly comparable with those for benzaldehyde and vanillin, (curves *C* and *B*, Fig. 1) where equimolar reaction mixtures were employed.

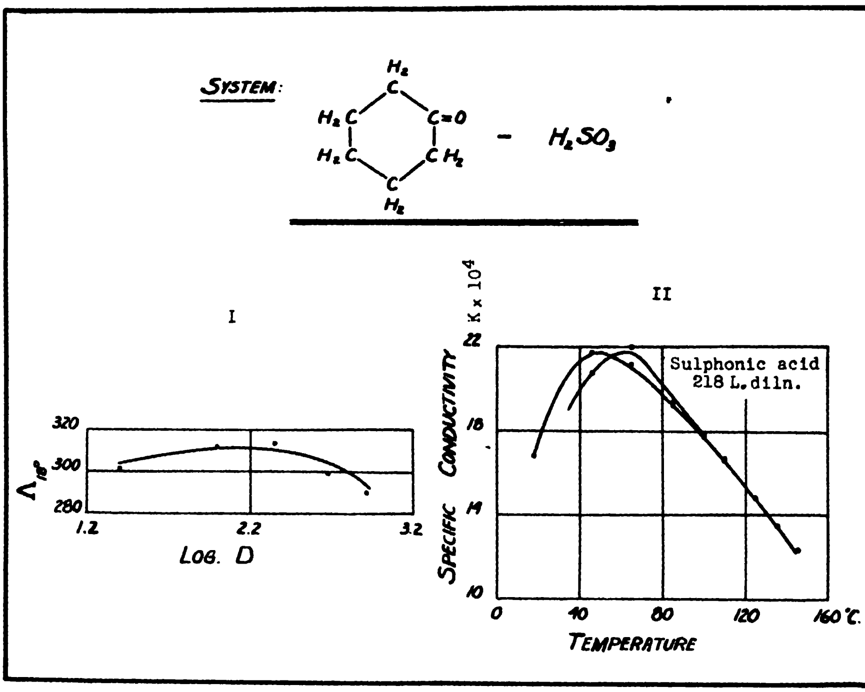


FIG. 4. *I. Equivalent conductivity of cyclohexanone sulphonic acid.*
II. Specific conductivity of cyclohexanone-sulphurous acid system.

In the actual order in which the work was experimentally carried out, this was the first sulphonic acid to show a comparatively mobile equilibrium and it was therefore considered necessary to determine whether the compound was a strong acid. The change in equivalent conductivity with dilution was studied at 18° C., the necessary free sulphurous acid corrections being obtained from the previous data of Hoover and Hunten (27). The resulting $\Lambda - \log D$ curve, Fig. 4, shows that the compound is a strong acid.

The change in specific conductivity with temperature is quite typical of saturated ketones, as shown in Fig. 4. Runs were made at dilutions of the order of 200 litres with 145° and 125° C. as respective maximum temperatures.

The data obtained indicated that the degree of permanent decomposition of the sulphonic acid at high temperatures depended on the extent and duration of the high temperature treatment, *i.e.*, with a lower maximum temperature or with a shorter time at high temperature (over about 110° C.), the original curve obtained during heating of the cell was more nearly repeated on cooling.

System:—Quinone—Sulphurous Acid

Quinone is rapidly and almost quantitatively reduced to hydroquinone with corresponding formation of sulphuric acid. A sulphonic acid is apparently not formed—in any event the possibility of its doing so is blocked by the rapidity of the other reaction.

System:—Resorcinol—Sulphurous Acid

A bisulphite derivative of resorcinol was prepared by Fuchs and Elsner (15). The reaction scheme postulated by them involved a primary conversion of resorcinol into its tautomeric alicyclic form, one mole of the latter then adding on three moles of the bisulphite.

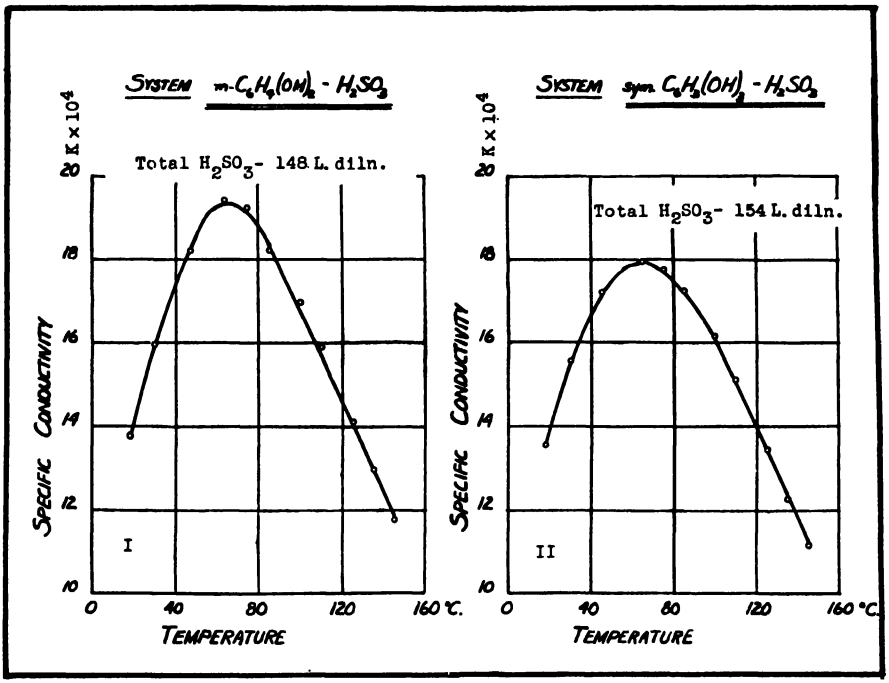


FIG. 5. *I. Specific conductivity of resorcinol-sulphurous acid system.*
II. Specific conductivity of phloroglucinol-sulphurous acid system.

Resorcinol, however, has been shown in the present investigation not to react with sulphurous acid between 18° and 145° C. No analytical combination was detectable at room temperature, and the conductivity curve (Fig. 5) is typical of sulphurous acid. The curve is strictly reproducible, the resorcinol preventing any oxidation of sulphurous acid. Any high temperature addition to an ethylene linkage formed by ketonization would be indicated on this curve.

This non-reactivity does not necessarily invalidate Fuchs and Elsner's results since in their experiments the more basic sodium bisulphite solution was employed. Even then their reaction was very slow indicating the small concentration of the keto form present at any time.

System:— Phloroglucinol—Sulphurous Acid

Fuchs (9) also investigated the reaction product of sodium bisulphite with ketonized phloroglucinol.

In the present study phloroglucinol was found to react very slightly with sulphurous acid to form the most unstable sulphonic acid yet encountered. The sulphonic acid was decomposed during iodometric titration of the free sulphurous acid even when an attempt was made to maintain the equilibrium in the presence of ice.

The conductivity-temperature curve is given in Fig. 5. The maximum is slightly less marked than that of the resorcinol curve indicating some combination. As in the case of resorcinol the curve obtained during cooling of the cell was identical with that for rising temperatures. The slight combination indicates phloroglucinol to be more easily ketonized than resorcin.

COMPOUNDS IN WHICH AN ETHYLENE LINKAGE IS THE ONLY REACTIVE GROUP

System:—Cinnamyl Alcohol—Sulphurous Acid

The interaction of cinnamyl alcohol and sodium bisulphite to form an unstable sodium sulphonate, $C_6H_5CH(SO_3Na)CH_2CH_2OH$, has been noted by Labbé (39). In this work it was found that if any reaction takes place between sulphurous acid and cinnamyl alcohol it does so very slowly even at 145° C. Analysis of the usual equimolar mixture showed no reaction in two days at room temperature. When heated in a sealed tube for 1 hr. at 145° C. the combined sulphurous acid amounted to 7.9% of the total, and in another sample heated for 6 hr. at 145° C., combination up to 15% took place. Although the tubes were flushed out thoroughly with nitrogen before loading it was practically impossible to prevent some air entering during sealing, so that even some of the observed combination may well be due to oxidation to cinnamic aldehyde by small amounts of enclosed oxygen, followed by combination of the aldehyde which would occur at once.

This was confirmed by conductivity measurements, which gave a sulphurous acid curve, Fig. 6. In the present instance, however, there is an objection to too strict a reliance on conductivity data. The solubility of the alcohol is very limited and as it was not possible to agitate the solution sufficiently during loading of the cell, nor to provide circulation in the cell itself, the liquid unquestionably contained considerable excess sulphurous acid. It is definitely established, however, that the presence of a carbonyl group in addition to an ethylene linkage greatly facilitates the addition of sulphurous acid to the latter and that therefore in the case of unsaturated aldehydes and ketones the —CO— sulphonic acid is probably an intermediate in the formation of the —C=C— acid.

System:— Tetrahydrobenzene—Sulphurous Acid

Tetrahydrobenzene has been shown to add on sulphurous acid at high

temperatures, giving a cyclohexane sulphonic acid.

After preliminary experiments had indicated that combination actually occurred, a number of sealed tubes, each containing 5 cc. of tetrahydrobenzene

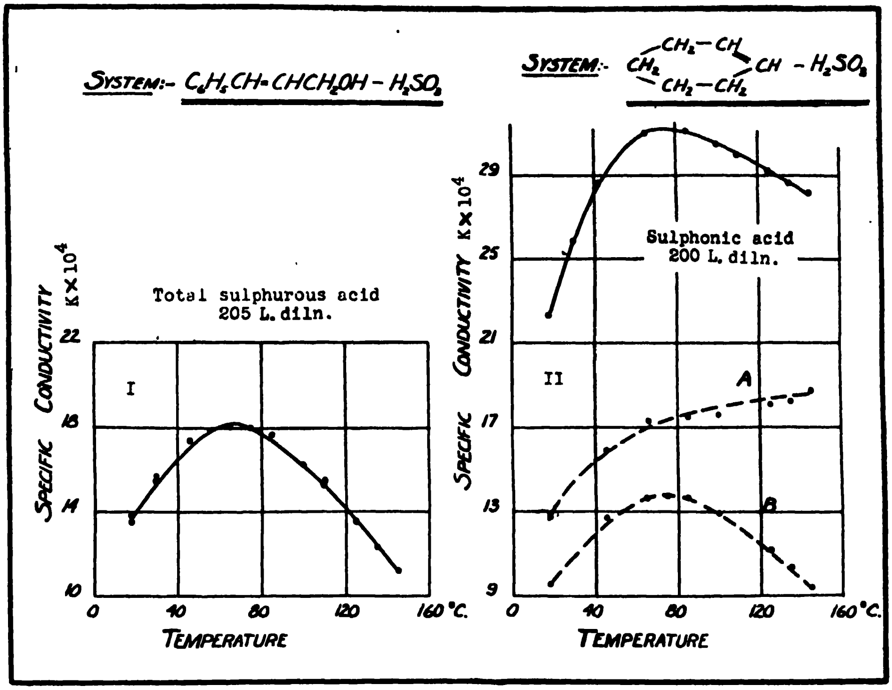


FIG. 6. *I. Specific conductivity of cinnamyl alcohol-sulphurous acid system. II. Specific conductivity of tetrahydrobenzene-sulphurous acid system. A. Specific conductivity of sulphonic acid (calc.). B. Specific conductivity of sulphurous acid. (Hoover and Hunten).*

and approximately 50 cc. of 0.1 *M* sulphurous acid were heated for one hour at 145° C. The tubes were flushed with nitrogen before loading and the heating time limited to one hour in order to obtain a solution for conductivity purposes as free as possible from sulphuric acid. The resulting liquid was poured into a nitrogen-filled flask and as much of the excess sulphurous acid as possible removed in vacuum. The mixture was then analyzed and a conductivity run made on a sample diluted to 200 litres total sulphurous acid. The data obtained are shown in Fig. 6 and are obviously the sum of two curves *A* and *B*, giving the conductivity of the sulphonic acid and that of the sulphurous acid present, respectively. It was possible to estimate the latter by interpolation between two curves for the conductivity of sulphurous acid obtained by Hoover and Hunten at two concentrations, one on either side of that shown by analysis to be present in the present instance, and hence to approximate to the true sulphonic acid curve by difference. The latter is seen to be the ethylene linkage type.

The rate of hydrolysis by barium hydroxide was also measured. Due to the extremely dilute character of the solutions used a considerable experimental

error was involved but the results indicated that no hydrolysis whatever took place as would be expected.

It is thus once again emphasized that sulphurous acid will only add on to an ethylene linkage very slowly and at high temperatures. The reaction with tetrahydrobenzene takes place much more readily than the corresponding reaction with cinnamyl alcohol (if indeed the latter takes place at all) but is still considerably more difficult to effect than when other groups are present which can react with sulphurous acid.

FURANE DERIVATIVES

The fact that α-furane derivatives show a certain degree of unsaturation and that the ring oxygen can form oxonium addition products has been recently emphasized by Moureu, Dufraisse and Johnson (41).

The interaction of furane derivatives and sulphurous acid was therefore considered as likely to be of interest and two such reactions were investigated; namely, those with furfuraldehyde and with furfuryl alcohol.

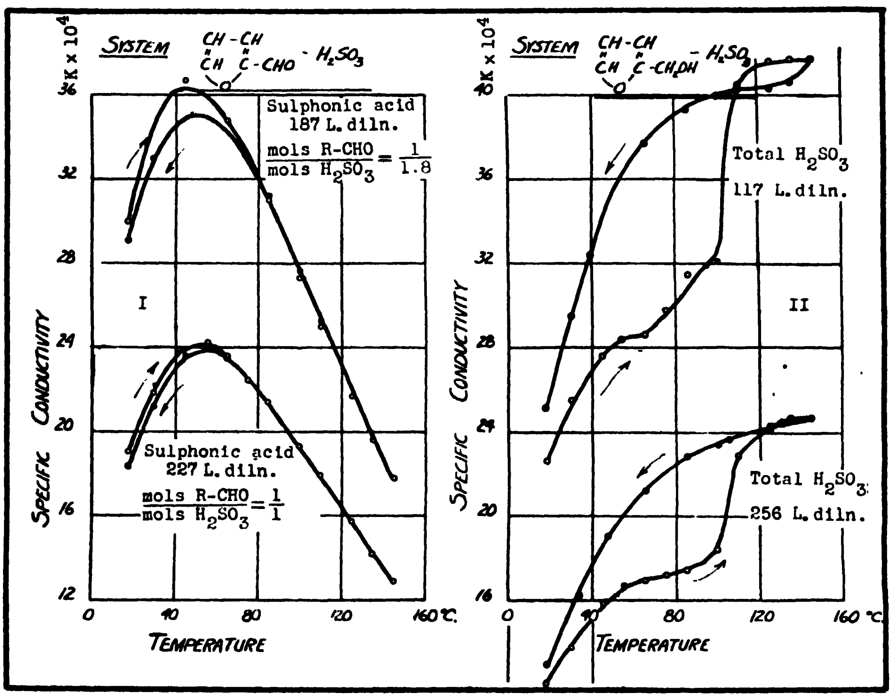


FIG. 7. *I. Specific conductivity of furfural-sulphurous acid system.*
II. Specific conductivity of furfuryl alcohol sulphurous acid system.

System:— Furfural—Sulphurous Acid

Furfural has been found to react like other nuclear aldehydes already studied (benzaldehyde and vanillin). The equilibrium of the usual reaction mixture was 88.4% on the side of the sulphonic acid (cf. benzaldehyde 92%, vanillin 80.7%). Furthermore the rate of hydrolysis with baryta was typically rapid, being complete in 10 min.

Conductivity-temperature curves, both for an equimolar mixture and with considerable excess sulphurous acid are shown in Fig. 7 and show that even in the presence of excess sulphurous acid no reaction with the furane ring takes place, and only the —CHO acid is formed.

System:— Furfuryl Alcohol—Sulphurous Acid

A gradual combination with sulphurous acid took place at room temperature. Chocolate-colored polymerization products were formed even in a nitrogen atmosphere. Conductivity-temperature curves, Fig. 7, were obtained the day after their respective reaction mixtures were prepared, and therefore before polymerization had set in to any extent, or the reaction had been completed. In both cases there was indicated definitely the formation of a sulphonic acid of the —C=C— type. This formation proceeds at a sufficient rate to show on the curves for temperatures above approximately 65° C. and appears to go extremely rapidly between 100 and 110° C. as indicated by the exceptionally rapid increase in conductivity over this range. On a second heating the down curve was reproduced.

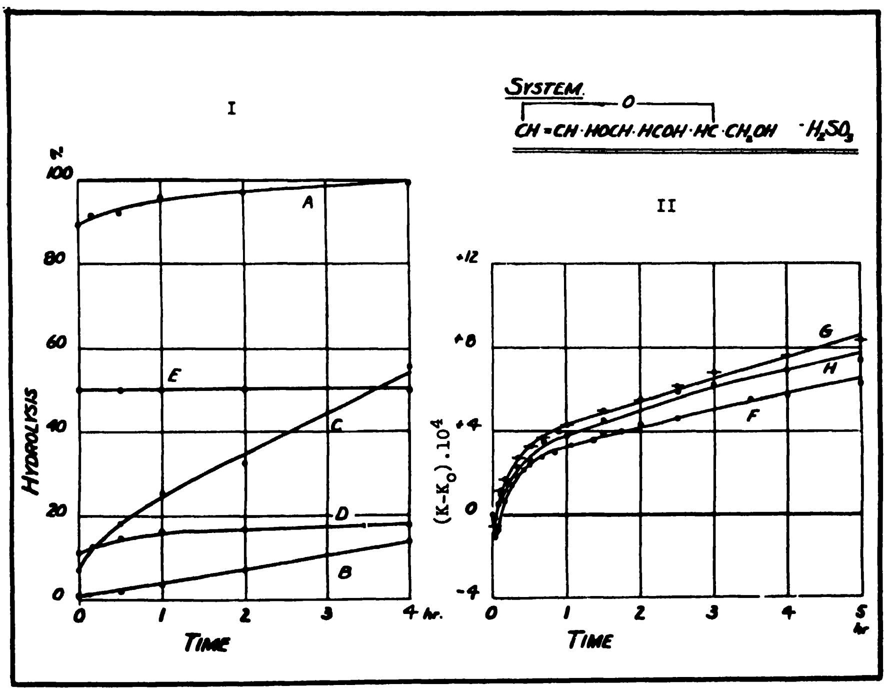
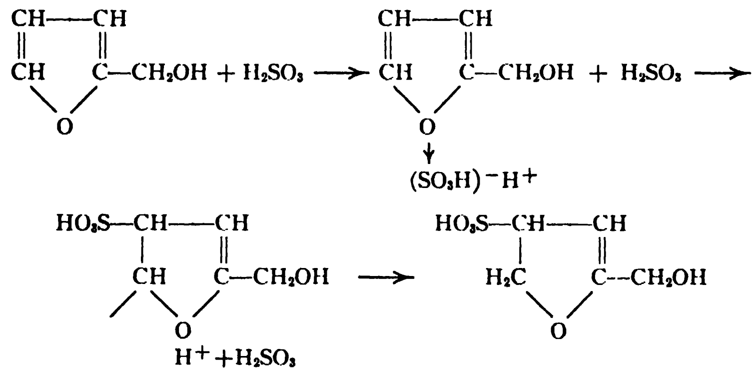


FIG. 8. *I. Hydrolysis of sulphonic acids with barium hydroxide. A. —CHO— acid of crotonaldehyde (0.0918 M). B. —C=C— acid of crotonaldehyde (0.0958 M) C. —C=C— acid of crotonaldehyde (0.0336 M). D. Furfuryl alcohol sulphonic acid (0.0678 M). E. Glucal sulphonic acid (0.0456 M). II. Rate of formation of —C=C— acid in glucal-sulphurous acid system. F. Temp. = 125° C. Cell heated rapidly. G. Temp. = 135° C. Cell heated rapidly. H. Temp. = 135° C. Cell held at 100° C. for ½ hr.*

Sealed tubes containing the reaction mixture were heated for 1 hr. at 110° C. to obtain the sulphonic acid for determination of the rate of hydrolysis with

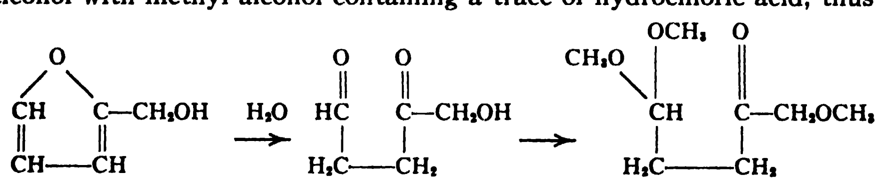
barium hydroxide. The resulting liquid was somewhat polymerized and on analysis showed that 82.3% of the total sulphurous acid was combined. The rate of hydrolysis with baryta is shown as curve *D* in Fig. 8. The sulphonic acid concentration was 0.0678 *M*. In the same figure, curve *A* gives the rate of hydrolysis for the —CHO reaction product of crotonaldehyde sulphonic acid [(CH_3—CH=CH—CH(OH)SO_3H)] at 0.0958 *M*, and curve *C* for the latter acid at 0.0336 *M*. On comparison with the other curves not only is the rate of hydrolysis seen to be very slow but the important point is at once brought out that practically all the hydrolysis that takes place, does so almost immediately. After the first few minutes the rate becomes almost negligible. Due to the insolubility of barium sulphite this cannot be due to the attainment of an equilibrium as would be the case if both reactants and reaction products were soluble but rather indicates that the sulphurous acid is combined in two different ways a small part being in unstable union and a larger percentage firmly bound.

Three possible mechanisms of reaction need to be considered in the light of these facts. The first involves the formation of an oxonium addition product, followed by entry of the sulphurous acid into the furane ring according to the scheme:



The second possibility is a scission of the furane ring, and the third a direct addition to the ethylene linkage.

In the second case, as shown by Pummerer and Gump (45), the first product would be δ-hydroxy-laevulinic aldehyde, $CH_2OH—CO—CH_2—CH_2—CHO$, since the dimethyl acetal of the latter was formed by treatment of furfuryl alcohol with methyl alcohol containing a trace of hydrochloric acid, thus



and yields of 40% laevulinic acid were obtained by heating furfuryl alcohol with 0.5% hydrochloric acid, or with 10% oxalic acid.

The experimental results, however, decidedly favor the first explanation. Thus it has been shown that the reaction takes place slowly at room tem-

perature, in approximately 0.1 *M* sulphurous acid. It hardly would be expected that scission of the ring would occur under these conditions. Secondly, the hydrolysis curve with baryta is typical of what would be expected if the first mechanism were true. Finally, the experimental data present a remarkable parallel with those observed for the pyrone derivative, glucal, which is discussed later and where conclusive evidence of the formation of an oxonium compound was obtained.

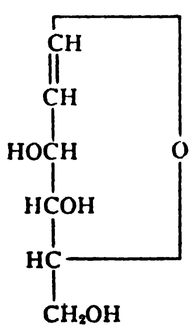
The fact that the product polymerizes very readily does not invalidate these conclusions since both furane and laevulinic aldehyde derivatives show this tendency to a marked degree. While the fact that furfuraldehyde does not show a similar reaction is indicative of some special mechanism like ring scission in the reaction of furfuryl alcohol with sulphurous acid, yet even this is discounted by the fact that furfural is one of the most stable of furane derivatives.

In view of the preceding work on unsaturated derivatives, direct addition to the ring is most unlikely. Even with tetrahydrobenzene such a reaction only takes place at high temperatures and furane derivatives show much greater saturation than this typically unsaturated compound.

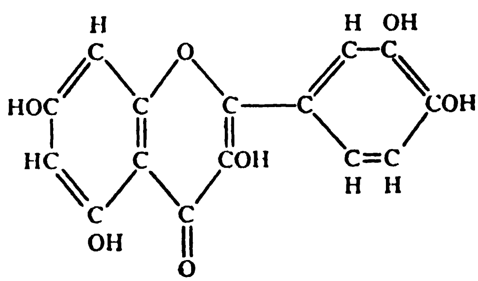
It is therefore concluded that, dependent on the other ring substituents, furane oxygen is capable of forming an oxonium addition product with sulphurous acid and that when such a compound is formed it can then serve as a medium for the introduction of the sulphonic acid group into the ring.

PYRONE DERIVATIVES

The pyrone derivatives studied in the present investigation were crystalline glucal III and the flavone derivative, quercetin IV.



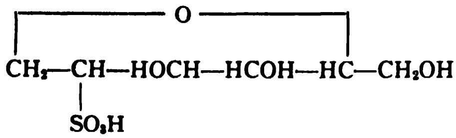
III



IV

System:— Glucal—Sulphurous Acid

Reaction took place slowly at room temperature. A conductivity-temperature curve was made on a mixture after two days standing (26% combined) at 204 litres total sulphurous acid dilution, Fig. 9. After passing through a sulphurous acid maximum, combination with the ethylene linkage was shown to take place at temperatures over 100° C. and this proceeded slowly and continuously, throughout the high temperature part of the cycle. Heating the



V

cell again gave the second curve indicating the formation of the —C=C— acid, presumably of structure V.

After five days standing at room temperature the sulphonic-acid content amounted to 56.7% of the total sulphurous acid and was raised to 75.4% by removing some excess sulphur dioxide in vacuum. Conductivity measurements, Fig. 9, showed that a comparatively unstable strong acid was present at low temperatures and was

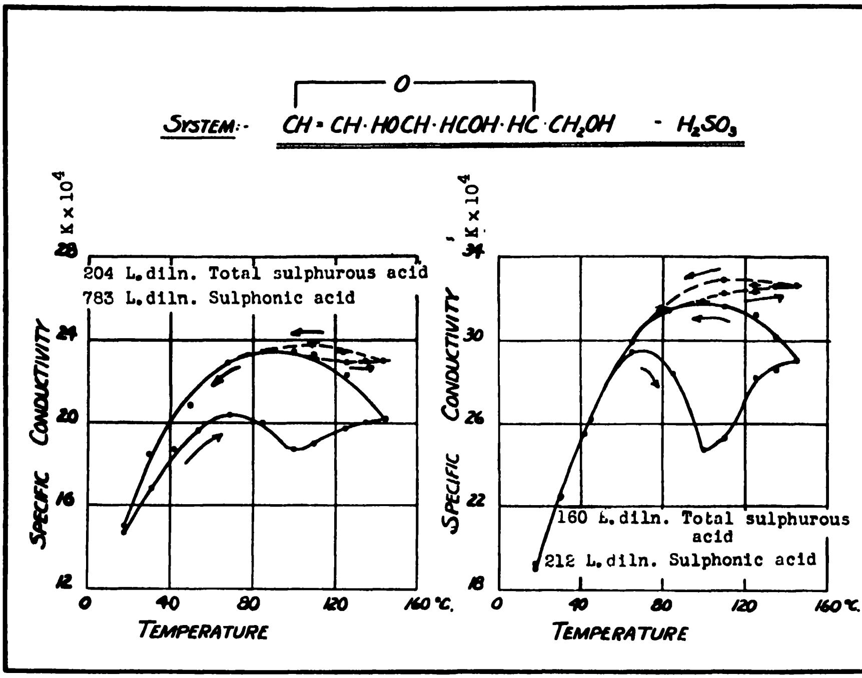
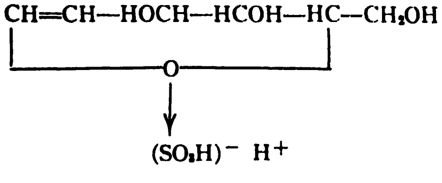


FIG. 9. *Specific conductivity of glucal-sulphurous acid system.*

decomposed on heating. At higher temperatures the —C=C— acid (V) was formed. The strength of the low temperature compound is shown by the fact that the specific conductivity at low temperatures was practically the same as that for the —C=C— acid. The decomposition is shown by the extreme character of the downward break above 65° C. On heating the cell a second time the —C=C— acid curve is obtained.

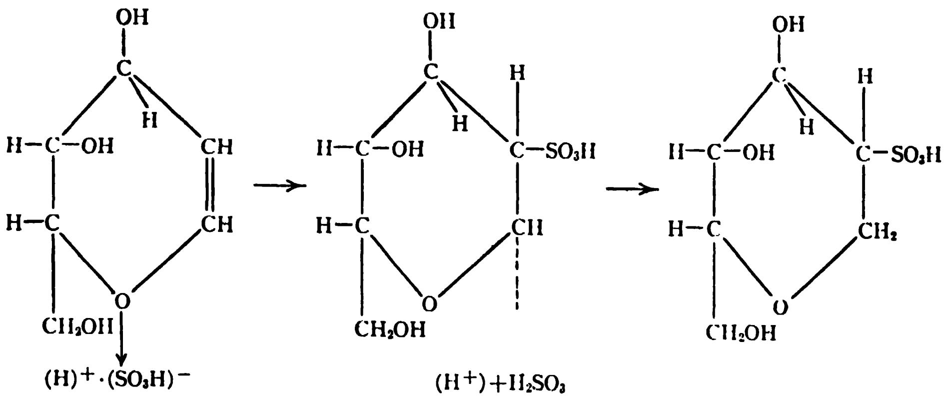
These facts, particularly the apparently high degree of dissociation and decomposition on heating, point strongly to the formation of an oxonium acid (VI) at low temperatures.

The data obtained when the reaction mixture was hydrolyzed with barium hydroxide are also in accord with these facts. Thus in the five-day reaction mixture (56.7% combined) 50% of the sulphonic acid was hydrolyzed imme-



VI

diately and none thereafter for any period up to four hours. This indicated that the —C=C— acid (V) was also present at room temperature and hence was formed through the mechanism involving the oxonium compound according to the scheme previously discussed for furfuryl alcohol, namely,



The mixture, heated for three hours with barium hydroxide, in a sealed tube at 145° C., turned a brownish color. Analysis showed that 86.9% of the total sulphurous acid was combined, and of this 3% was immediately hydrolyzable, the remainder being unaffected for periods of hydrolysis up to four hours, thus confirming the high temperature formation of the —C=C-- acid.

The change in specific conductivity with time was measured at critical temperatures, Fig. 8. For curve *F* the cell was heated rapidly to 125° C and held at that temperature. Curve *G* was obtained similarly at 135° C. For curve *H* the cell was heated to 100° C., held there for ½ hr. to permit decomposition of the low temperature reaction product and then heated to 135° C. (zero time). All three curves showed a slight fall in specific conductivity after reaching the final temperature indicating in every case incomplete decomposition of the oxonium compound when the final temperature was reached. The effect of holding the cell at 100° C. for ½ hr. is shown by the lower fall in conductivity in curve *H*. In spite of the fact, however, that curve *G* initially shows a lower (algebraic) change in conductivity with time, it shortly crosses curve *H*, which is for the same temperature, and eventually flattens out so that *G* and *H* become nearly parallel as shown in the figure. The only reason for any difference is that for curve *G* there was a higher concentration of the low temperature reaction product at zero time. Since the rate of formation of the —C=C— acid is increased by increased concentration of the supposed oxonium compound it would seem to be conclusively demonstrated that the latter is an intermediate in the reaction mechanism by which

the more stable compound is formed. Strong evidence in favor of a scheme such as that given previously is thus obtained.

Curve F at 125° C. shows a much greater initial fall in conductivity than either of the 135° C. curves. Although the zero time concentration of the supposed oxonium compound is greater (since the temperature is lower) the initial rate of formation of the —C=C— acid is slower than for curve G. It may thus be assumed that for equal concentrations of the oxonium compound the rate of formation of the more stable acid increases with rising temperature, which is quite in accord with expectations from the suggested mechanism. After the large initial drop in conductivity the reaction rate becomes nearly the same as that for curves G and H (the curves are nearly parallel) so that the loss due to lower temperature must be compensated for to a large extent by increased concentration of the oxonium compound. This is further evidence in favor of the proposed mechanism involving the consecutive reactions: glucal + sulphurous acid $\rightleftharpoons$ oxonium derivative; oxonium derivative $\longrightarrow$ —C=C— sulphonic acid, since the equilibrium given by the first equation would be shifted to the left by increasing temperature.

A certain amount of direct addition to the ethylene linkage is also possible at the higher temperatures, analogous to that observed for tetrahydrobenzene. It would seem however that if direct addition does occur, the amount of stable acid so formed is relatively small up to about 135° C.

The only unstable reaction products other than oxonium derivatives would involve ring scission and contain sulphur linked through oxygen to carbon such as the compound VII. Any such products would be most unlikely. The stability of the pyrone ring in glucal is of course directly bound up with the ethylene linkage between the 1- and 2-carbon atoms and is of the same essential character as the pyrone ring in flavones and flavonols.

$$\mathrm{HOCH{=}CH{-}HOCH{-}HCOH{-}\underset{\substack{|\\ O-SO_2H}}{HC}{-}CH_2OH}$$

VII

The experimental facts are best explained by the assumption of the formation of an oxonium derivative of the type postulated and that the subsequent change to a more stable acid at a high temperature takes place through the mechanism suggested.

System:—Quercetin—Sulphurous Acid

Quercetin reacted extremely slowly due, at least in part, to its slight solubility. Even when excess sulphur dioxide was removed in vacuum the combined molarity could not be increased above 38% of the total theoretical amount since decomposition of the sulphonic acid resulted in further removal of sulphur dioxide.

A conductivity-temperature curve for a suitably diluted sample of this maximum combined liquid, Fig. 10, showed no formation of a —C=C— type acid at high temperatures. The probable reason for this is seen when a reaction scheme analogous to that for glucal is drawn up. The oxonium derivative

would be that indicated by (VIII) and the product formed by entry into the ring by (IX).

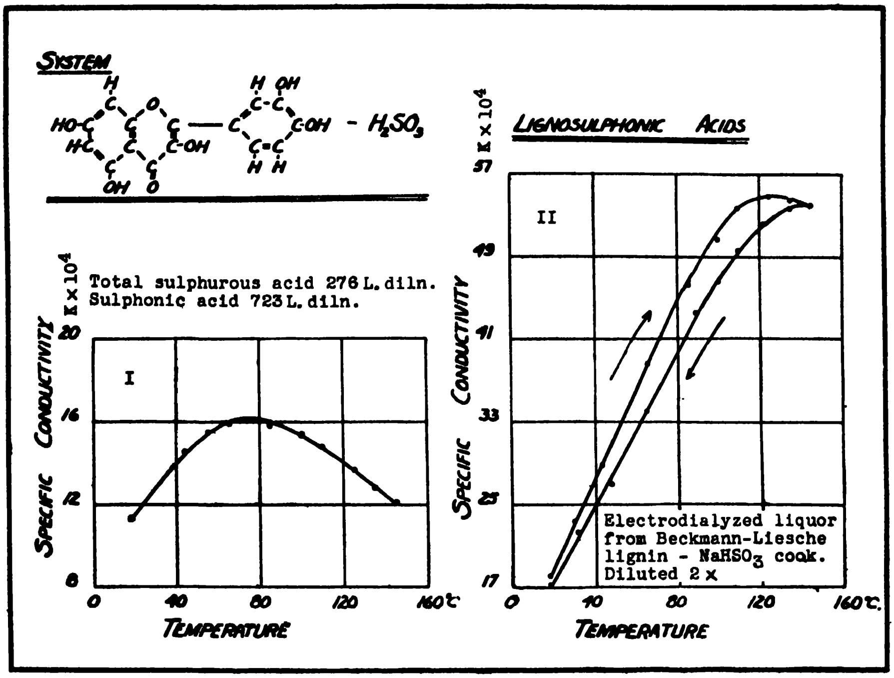
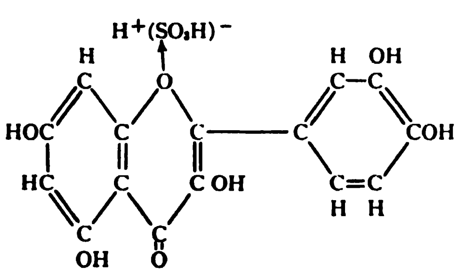
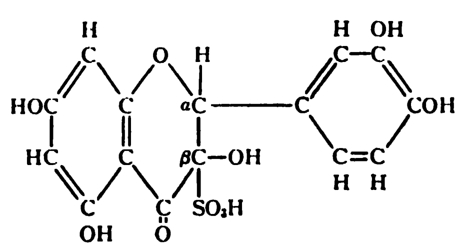


FIG. 10. *I. Specific conductivity of quercetin-sulphurous acid system. II. Specific conductivity of lignosulphonic acid from Beckmann-Liesche-Lehmann lignin.*

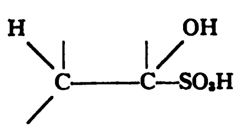


VIII



IX

The characteristic flavonol hydroxyl (on the β-carbon) makes the acid IX similar to that which would be formed by a cyclic ketone such as cyclohexanone since it contains the grouping (X); hence the acid should show ketone sulphonic acid characteristics and the conductivity curve which at first appears contradictory is thus brought into accord with the results previously obtained. This is further supported by the fact that hydrolysis with baryta is complete almost at once while the slow reaction rate and the final low concentration of sulphonic acid would be similarly explained by the theory.



X

The matter cannot be considered as definitely proved however until a derivative of the flavone type is investigated. If the latter reacts similarly to glucal the theory will be completely substantiated and an interesting essential difference between flavones and flavonols emphasized. In the meantime however the alternative possibility of slow hydrolysis of the flavonol derivative with the production of a keto hydroxy body must also be considered.

Lignosulphonic Acids

The main difficulty in studying the interaction of lignin with sulphurous acid is that the lignin isolated by any of the known methods is so changed during the process of its separation from the wood that it will not combine with free sulphurous acid even at high temperatures. Three sources of lignin were used in this investigation, namely, (a) the liquor produced by heating in a glass digester in a nitrogen atmosphere a mixture of sulphurous acid and spruce meal previously extracted with benzene-alcohol; (b) waste sulphite liquors obtained from the usual commercial processes of sulphite cooking; and (c) lignin isolated from straw by the Beckmann-Liesche-Lehmann method (1) and which was heated in sealed tubes with sodium bisulphite solution. The free sulphonic acids were in each case obtained by electrodialysis of the appropriate liquid.

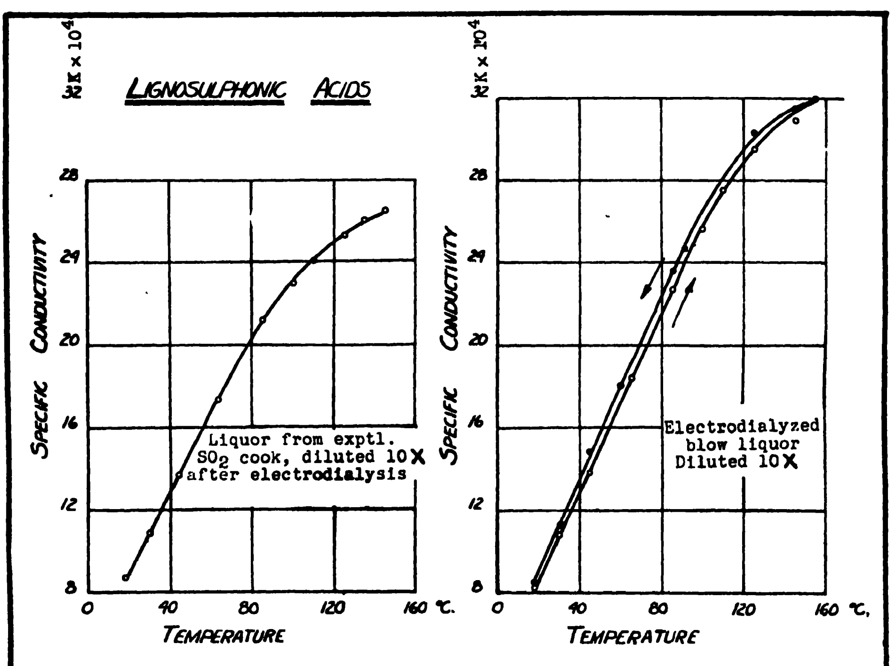


FIG. 11. *Specific conductivity of lignosulphonic acids.*

The products from the last two processes still contained a considerable amount of inorganic matter as shown by their ash content. In fact the electrodialysis of the waste liquors, although decreasing the quantity of ash,

brought about an increase in the ratio of ash to total solids. This is in accord with the results of other investigators (21), who found that the free acid always contained considerable amounts of mineral matter, and indicates something of the nature of inner complex salt formation by the high molecular complexes present.

Conductivity-temperature curves for the electrodialyzed liquors after suitable dilution are plotted in Fig. 10 and 11. It must be specifically emphasized that the absolute values of the conductivity mean very little and that the important point is the type of curve,—namely, one showing continually increasing conductivity with rise in temperature. It is thus established that in the final product the sulphonic acid group is attached to a —C=C— linkage of some sort. The curves give no indication as to the mechanism of formation, nor are any claims made as to the homogeneity of the products. The data submitted previously, however, indicate strongly that, besides an ethylene linkage either a carbonyl group, or a ring oxygen capable of oxonium salt formation, or both, play a role in the reaction. This point of view is supported by other evidence which points to the formation of an oxonium compound as an intermediate compound and which is discussed in the succeeding paragraphs.

Owing to the fact that some compounds present exert a buffer action it is not possible to determine accurately the total acidity of the sulphite liquors. For this reason the hydrolysis with barium hydroxide cannot be calculated as a "per cent hydrolysis" according to the usual method. Iodometric titrations can be run, however, and a comparison of the value obtained for iodine absorption with reaction time is obviously comparable with the usual baryta hydrolysis curves. Such data, obtained on the fresh sulphite liquor from the sulphurous acid reaction products showed some increase in sulphurous acid content immediately after treatment with barium hydroxide and none thereafter. The same liquors after standing for several days, and also the waste sulphite liquors, showed no increase whatever, a phenomenon somewhat similar to that observed with furfuryl alcohol and glucal.

This phenomenon, supposedly indicative of an unstable reaction product, has been noted by other workers. Thus Klason (30, 31, 36) concluded that sulphurous acid forms a stable chemical compound with lignin but that a certain amount of unstable compound is also produced. Samec and Rebek (47) showed that during dialysis, sulphite liquor loses sulphites which are "loosely bound" to the lignin and which they say are joined to carbonyl groups. Sulphite, bound as oxonium compound, would of course be even more easily removable than that attached to a carbonyl group. The latter would be decomposed only by removal of free sulphurous acid and consequent equilibrium shift. Furthermore, the general conception of the instability of carbonyl derivatives seems to be based on the erroneous notion that these compounds are sulphite esters and not sulphonic acids. This is shown by Fuchs' formulation of the reaction products of sodium bisulphite with the tautomeric alicyclic forms of resorcinol and phloroglucinol already mentioned.

When the reaction liquid obtained by heating Beckmann-Liesche-Lehmann lignin with sodium bisulphite was allowed to stand, or was electrodialyzed, a

precipitate was formed. This contained no sulphur and was not unlike the original lignin in appearance. Since it was formed in the presence of a marked excess of sulphite it may well have resulted from decomposition of an oxonium derivative. Its appearance in the middle compartment near the anode membrane of the electrodialysis apparatus suggests that it was not, like the precipitation on the anode in an outer compartment, of sufficiently small dimensions to pass through the parchment.

This mechanism of sulphonic acid formation would explain very reasonably the experimental conditions actually involved in the commercial manufacture of sulphite pulp. The fact that the digester must be held at 110° C. for some time before heating to a higher temperature would be a necessary deduction from a mechanism involving an oxonium derivative. Comparatively rapid combination with the lignin could only occur through the medium of oxonium addition. The addition product would be decomposed on heating to higher temperatures and the reaction therefore largely prevented. Also since the formation of the —C=C— acid in the case of glucal was shown to take place at a comparatively rapid rate only at 110° C. and higher, this is indicated as a highly probable critical temperature for a proper balance between the decomposition of the oxonium derivative and the formation of a —C=C— acid.

At this temperature direct addition to the ethylene linkage is also possible but should proceed very slowly. The fact that the formation of the —C=C— acid of crotonaldehyde ($CH_3CH(SO_3H)CH_2CHO$) is apparently autocatalytic (27) is of interest in this connection, since it is known that sulphite cooking is aided by the addition of spent liquor from a previous cook, and practically all the commercially used acid contains a certain amount of spent liquor.

In the authors' opinion therefore the formation of lignosulphonic acids probably takes place primarily through the medium of an oxonium addition product and to a very much lesser extent by direct addition to an ethylene linkage. The latter process proceeds very slowly but may become the main reaction during the later stages of the cooking. The former represents the mechanism by which the greater part of the lignosulphonic acid formation occurs and is particularly effective during the early stages.

The results of the present investigation also provide further and more convincing evidence for the existence of both a ring oxygen and an ethylene linkage in the same ring structure in the lignin molecule.

This investigation also shows that the suggested formation of the lignosulphonic acids by ketonization of a phenolic nucleus is most unlikely, and that the existence of a carbonyl group is not necessary for the formation of these acids or of the loosely bound sulphonic acids noted by other investigators.

Experimental Part

A detailed description of the apparatus used, the method of measuring specific conductivities, and the methods of analysis employed have been given in a previous communication (27).

System:— Crotonaldehyde—Sulphurous Acid

Crude crotonaldehyde was treated with anhydrous sodium carbonate to

TABLE I

HYDROLYSIS OF CROTONALDEHYDE SULPHONIC ACIDS

Agent	Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed M/litre	R—SO_3H hydrolyzed %
$CH_3CH{=}CH{-}CH(OH)SO_3H$. Acid: total, 0.1022$M$; free, 0.0104$M$; combined, 0.0918$M$.					
Ba(OH)$_2$ sat'd. sol'n., 20cc.	At once	18.41	0.0921	0.0817	89.1
	10	18.79	0.0940	0.0836	91.2
	30	18.94	0.0947	0.0843	91.8
	60	19.68	0.0984	0.0880	95.8
	120	19.84	0.0992	0.0888	96.7
	240	20.21	0.1011	0.0907	98.8
$CH_3CH{=}CH{-}CH(OH)SO_3H$. Acid: total, 0.0371 M; free, 0.0042M; combined, 0.0329M.					
Ba(OH)$_2$ sat'd. sol'n., 20cc.	At once	6.16	0.0308	0.0266	80.9
	10	6.48	0.0324	0.0282	85.7
	30	6.80	0.0340	0.0298	90.7
	60	7.17	0.0358	0.0316	96.1
	120	7.33	0.0367	0.0325	98.8
	240	7.44	0.0372	0.0330	100
$CH_3CH(SO_3H)CH_2CHO$. Acid: total, 0.1012M; free, 0.0054M; combined, 0.0958M.					
Ba(OH)$_2$ sat'd. sol'n., 20cc.	At once	1.27	0.0064	0.0010	1.0
	30	1.44	0.0072	0.0018	1.9
	60	1.74	0.0087	0.0033	3.5
	120	2.61	0.0121	0.0067	7.0
	240	3.71	0.0186	0.0132	13.8
$CH_3CH(SO_3H)CH_2CHO$. Acid: total, 0.0339M; free, 0.0003M; combined, 0.0336M.					
Ba(OH)$_2$ sat'd. sol'n., 20cc.	At once	0.45	0.0028	0.0025	7.4
	10	0.92	0.0046	0.0043	12.8
	30	1.28	0.0064	0.0061	18.1
	60	1.77	0.0089	0.0086	25.6
	120	2.26	0.0113	0.0110	32.7
	240	3.78	0.0189	0.0186	55.3
$CH_3CH{=}CH{-}CH(OH)SO_3H$. Acid: total, 0.0312$M$; free, 0.0002$M$; combined, 0.0310$M$.					
NaOH	10	0.22	0.0011	0.0009	% decr. 1-3 constant within limits of exptl. error
	30	0.16	0.0008	0.0006	
	60	0.10	0.0005	0.0003	
	120	0.16	0.0008	0.0006	
	240	0.10	0.0005	0.0003	
$CH_3CH_2CH(SO_3H)CHO$. Acid: total, 0.0312 M; free, 0.0002M; combined, 0.0310M.					
NaOH	10	0.16	0.0008	0.0006	% decr. 1-2 constant within limits of exptl. error
	30	0.16	0.0008	0.0006	
	60	0.16	0.0008	0.0006	
	120	0.10	0.0005	0.0003	
	240	0.10	0.0005	0.0003	

NOTE:— *Temperature 20° C.; volume of liquid, 10 cc.*

neutralize crotonic acid and remove water, and then fractionally distilled. This process was repeated three or four times until a product of b.p. 103-104° C. was obtained. The aldehyde was used immediately after the final distillation.

The conductivity data for this system have been given (27).

The rate of hydrolysis with barium hydroxide of both the crotonaldehyde sulphonic acids was measured. The hydrolyses were carried out in 250-cc. ground glass stoppered bottles which had been thoroughly flushed out with nitrogen. In the usual procedure 10 cc. of the reaction mixture and 20 cc. of a saturated solution of barium hydroxide at 20° C. were allowed to react at 20° C. in a water thermostat for the required time, and the hydrolysis then stopped by rapid addition of a mixture of 20 cc. standard iodine (approx. $N/10$) and 10 cc. of 1:1 hydrochloric acid. The excess iodine was titrated with thiosulphate.

The —CHO acid (addition to aldehyde group) was obtained from the usual reaction mixture and the —C=C— acid (addition to —C=C— group) by heating the former in sealed tubes at 110° C. in an air furnace for one hour. The experiments were carried out for each acid at two different sulphonic acid molarities.

The mechanism of this hydrolysis was shown to be an equilibrium shift by repeating the hydrolysis experiments using sodium hydroxide instead of barium hydroxide and at the same normality and with the same experimental procedure. The results are given in Table I.

System:— Benzaldehyde—Sulphurous Acid

Kahlbaum's benzaldehyde was purified by washing with 1% sodium carbonate solution and then with distilled water (oxygen free), extracting with ether, drying over anhydrous sodium sulphate, decanting and distilling off the ether in a nitrogen atmosphere. Analysis of equimolar mixture:— Sulphurous acid: total, 0.1121 M; free, 0.0092 M; sulphonic acid, 0.1029 M (91.6% combined).

TABLE II

EFFECT OF DILUTION ON THE EQUILIBRIUM

Dilution calculated, litres	100	200	400	800
Dilution analyzed, litres	116	229	474	943

NOTE:— *Original reaction mixture; 0.1029 M sulphonic acid.*

TABLE III

RATE OF HYDROLYSIS OF BENZALDEHYDE SULPHONIC ACID WITH BARIUM HYDROXIDE

Time min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	21.98	0.1099	0.1010	97.8
60	22.38	0.1119	0.1030	99.8

NOTE:— *Sulphurous acid: total, 0.1121 M: free, 0.0089 M: sulphonic acid, 0.1032 M. Temp., 20° C.*

The sulphonic acid is decomposed with equilibrium shift on removal of free sulphurous acid. Analyses for the latter have to be carried out with ice in the analyzing mixture. The equilibrium is also shifted on dilution as is shown by Table II.

TABLE IV

SPECIFIC CONDUCTIVITY OF BENZALDEHYDE-SULPHUROUS ACID SYSTEM

Sulphonic acid 229 litres dilution				Sulphonic acid 227 litres dilution			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	185.4	145	144.4	18	180.8	110	190.2
30	220.0	135	159.4	30	218.5	100	207.5
45	246.5	125	175.6	45	246.5	85	235.5
65	251.8	110	202.1	55	251.8	65	268.3
75.6	243.5	100	219.9	65	281.8	58	272.2
85	231.3	85	248.1	85	230.9	45	266.5
100	209.9	65	278.5	100	205.3	30	231.0
110	194.2	45	289.8	110	190.2	18	200.5
125	171.4	30	255.3				
135	155.1	18	225.5				
145	144.4						

System:— Vanillin—Sulphurous Acid

Vanillin (Antoine Chiris, U.S.P. IX) was purified by recrystallization from aqueous alcohol, m.p. 80-81° C. After 24 hr. the equimolar mixture with sulphurous acid analyzed:— sulphurous acid: total 0.1120 M; free, 0.0214 M: sulphonic acid, 0.0906 M (or 80.7% combined). In the determination of free sulphurous acid, ice had to be used to freeze the equilibrium.

TABLE V

EFFECT OF DILUTION ON THE EQUILIBRIUM

Dilution calculated, litres	200	400
Dilution analyzed, litres	260	513

TABLE VI

HYDROLYSIS OF VANILLIN SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	21.06	0.1053	0.0825	96.8
60	21.31	0.1066	0.0838	98.8

NOTE:— *Sulphurous acid; total, 0.1070M; free, 0.0228M: sulphonic acid, 0.0842 M.*

TABLE VII

SPECIFIC CONDUCTIVITY OF VANILLIN-SULPHUROUS ACID SYSTEM

R—SO_3H 260 litres diln. =64.0% total H_2SO_3		R—SO_3H 256 litres diln. =64.1% total H_2SO_3		R—SO_3H 523 litres diln. =59.2% total H_2SO_3	
Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$
18	193.1	18	197.0	18	104.3
25	212.1	49.7	241.9	30	122.9
34.5	229.6	65	240.0	45	137.1
44.5	238.5	85	223.4	65	142.7
55.5	241.9	100	206.0	85	141.6
71.5	235.8	110	197.1	100	136.2
85	225.5	100	212.1	110	130.9
100	209.8	85	235.8	125	119.2
110	196.2	75	248.0	135	110.2
125	176.2	65	259.0	145	102.0
135	162.1	55	268.4	135	112.8
145	148.2	45	264.5	125	122.9
135	163.8	30	241.9	110	136.8
125	180.7	18	212.1	100	146.0
110	204.0			81	158.1
100	220.0			65	164.7
82	243.5			45	163.8
72	257.2			30	144.2
65	262.5			18	126.2
53	268.4				
41	268.4				
34.5	257.2				
25	240.1				
18	221.5				

System:— Glucose—Sulphurous Acid

Schering-Kahlbaum's "purest anhydrous" dextrose was used. The equimolar reaction mixture with sulphurous acid after standing for five days analyzed:— sulphurous acid: total, 0.0810 *M*; free, 0.0766 *M:* sulphonic acid, 0.0044 *M* (or 5.4% combined). A sample was transferred from the reaction vessel to a flask filled with nitrogen and excess sulphur dioxide removed with the suction pump. Analysis of resulting liquor:—sulphurous acid; total, 0.0215 *M;* free, 0.0183 *M:* sulphonic acid; 0.0032 *M* (or 14.9% combined). This was considered to at least approximate to the final equilibrium since some of the sulphonic acid had been decomposed.

TABLE VIII

SPECIFIC CONDUCTIVITY OF GLUCOSE-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling			
Temp.°C.	$k.10^6$	Temp.°C.	$k.10^6$	Temp.°C.	$k.10^6$	Temp.°C.	$k.10^6$
18	452	100	393	145	255	65	487
43	514	110	359	135	283	46	497
55.5	524	125	312	125	313	30	460
65.8	495	135	283	100	398	18	399
85	444	145	255	85	446		

NOTE:— *Sulphonic acid = 312 litres dilution = 14.9% of total sulphurous acid present (i.e., sulphurous acid = 54.7 litres dilution).*

System:—Methyl Ethyl Ketone—Sulphurous Acid

Eastman's product was purified by distillation; b.p. 80-81° C. Analysis of an equimolar reaction mixture after 24 hr. standing:— sulphurous acid: total, 0.1575 *M*; free, 0.0878 *M*: sulphonic, 0.0697 (44.3% combined). After addition of more ketone (five times excess) analysis gave: sulphurous acid; total, 0.1575 *M*; free, 0.0307 *M*: sulphonic acid, 0.1268 *M* (80.5% combined).

TABLE IX

EFFECT OF DILUTION ON THE EQUILIBRIUM

Dilution calculated, litres	100	200	400
Dilution analyzed, litres	120	278	603

NOTE:—*Original reaction mixture—moles ketone: moles H_2SO_3 : : 5:1, with sulphonic acid, 0.1268 M.*

TABLE X

SPECIFIC CONDUCTIVITY OF METHYL ETHYL KETONE-SULPHUROUS ACID SYSTEM

R—SO_3H 120 litres diln. =70.7% total H_2SO_3		R—SO_3H 278 litres diln. =61.3% total H_2SO_3		R—SO_3H 603 litres diln. =57.4% total H_2SO_3	
Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$
18	362.1	18	183.8	18	93.2
25	381.8	25	203.4	25	103.8
35	395.4	35	223.0	35	115.3
45	389.5	44.5	219.8	45	122.2
55	375.3	55	222.1	54.5	125.3
65	358.2	65	219.8	65	127.7
85	320.3	85	209 0	85	127.2
100	285.0	100	194.6	100	124.2
110	262.5	110	182.3	110	117.9
125	229.8	125	160.8	125	106.9
135	207.8	135	146.8	135	98.2
145	185.7	145	127.9	145	90.6
125	229.8	135	147.8	135	98.4
100	285.0	125	160.0	125	107.1
65	360.5	85	212.6	100	125.0
44.5	387.5	65	225.8	67.5	135.8
35	378.9	46.5	223.9	45	125.0
25	360.5	34.5	211.6	35	116.8
18	343.9	24.5	194.8	25	106.2
		18	181.2	18	96.8

NOTE:— *Moles ketone: moles H_2SO_3 : : 5:1.*

System:— Benzylidene Acetone—Sulphurous Acid

Kahlbaum's product was recrystallized from benzene, m.p. 30-31° C. Equilibrium with sulphurous acid was only slowly attained. Analysis of usual equimolar mixture after three days standing gave:— sulphurous acid: total, 0.0642 *M*; free, 0.0389 *M*: sulphonic acid, 0.0253 *M* (39.4% combined). The mixture still contained undissolved ketone.

The following day excess sulphur dioxide was removed in vacuum, giving a liquid analyzing:— sulphurous acid: total, 0.0381 *M*; free, 0.0060 *M*: sulphonic acid, 0.0321 *M* (84.3% combined).

TABLE XI

SPECIFIC CONDUCTIVITY OF BENZYLIDENE ACETONE-SULPHUROUS ACID SYSTEM

R—SO_3H 154 litres diln. 79.2% of total H_2SO_3				R—SO_3H 397 litres diln. 83.7% of total H_2SO_3			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	259	145	553	18	105	145	255
30	304	135	547	30	127	134.5	250
48	369	123.1	544	45	153	125	248
65	417	110	533	65	180	110	243
75	440	100	524	75	192	100	238
85	465	83	500	85.1	205	65	214
100	493	65	462	100	219	45	188
110	507	44.5	406	110	228	30	161
125	537	30	361	125	238	18	136
135	544	18	308	135	248		
145	553			145	255		

TABLE XII

HYDROLYSIS OF BENZYLIDENE ACETONE SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	1.20	0.0060	0.0016	4.9
10	2.68	0.0134	0.0090	36.4
30	5.02	0.0251	0.0207	64.0
60	6.18	0.0309	0.0265	80.8
120	7.08	0.0354	0.0310	94.6
210	7.40	0.0370	0.0326	99.4

NOTE:— *Sulphurous acid: total, 0.0372 M; free, 0.0044 M: sulphonic acid, 0.0328M—volume of reaction mixture, 10 cc.*

System:—Dibenzyl Ketone—Sulphurous Acid

Eastman's "practical" grade was purified by washing with 1% sodium bisulphite, then with water, extracting with ether, drying over anhydrous sodium sulphate and recrystallizing from anhydrous ether; pale yellow needles, m.p. 29-30° C.

The product does not react with sulphurous acid under the experimental conditions studied. In equimolar mixture aqueous and non-aqueous layers result, both of which were analyzed. The micro sulphur analyses of the solutions were carried out with standard microanalytical apparatus according to the method of Pregl (44).

(1) Volumetric analysis of aqueous layer:—sulphonic acid: total, 0.0758 *M;* free, 0.0756 *M.* (2) Micro sulphur analysis of aqueous layer:— found: $BaSO_4$ per cc.; 17.38, 17.63 mg.; av., 17.50 mg. = 0.0750 *M*, *i.e.*, the same as the volumetric analysis for H_2SO_3. (3) Micro sulphur analysis of non-aqueous

layer:— found; $BaSO_4$ per cc.; 1.0, 1.3 mg., a quantity attributable to wetting by aqueous layer and indicating no combination. Dibenzyl ketone (2 gm.) heated with 40 cc. 0.1 *M* sulphurous acid in a sealed tube at 145° C. for 2 hr., after cooling, (aqueous layer) showed:— sulphurous acid: total, 0.0958 *M*; free, 0.0957 *M*, *i.e.*, no reaction over range studied.

System:— Dibenzoyl Methane—Sulphurous Acid

Dibenzoyl methane was purified by recrystallization from ligroin (60-70° C.); reddish-pink flat plates, m p. 77-78°C.

The compound (1 gm.) was heated for two hours at 145°C. in a sealed tube (nitrogen atmosphere) with 40 cc. of approximately *M*/10 sulphurous acid. The aqueous layer on cooling analyzed:— sulphurous acid: total 0.0978 *M*; free, 0.0978 *M*, showing no combination.

System:— Cyclohexanone—Sulphurous Acid

Kahlbaum's product was purified by redistillation, b.p. 155.5-156.5° C. The equimolar mixture with sulphurous acid gave:— sulphurous acid: total, 0.1156 *M*; free, 0.0402 *M*: sulphonic acid, 0.0754 *M* (65.2% combined). More cyclohexanone was added to shift the equilibrium towards the side of the sulphonic acid. With 2½ moles of ketone per mole of sulphurous acid, analysis showed:— sulphurous acid: total: 0.1111 *M*; free, 0.0053 *M*: sulphonic acid, 0.1058 *M* (95.0% combined).

TABLE XIII

EFFECT OF DILUTION ON THE EQUILIBRIUM

Dilution calculated, litres	24.5	100	200	400	800
Dilution analyzed, litres	25.0	108	219	421	835

NOTE:— *Original reaction mixture; 0.1058 M sulphonic acid; moles ketone: moles H_2SO_3 : : 2.5:1.*

TABLE XIV

EQUIVALENT CONDUCTIVITY AT 18°C. OF CYCLOHEXANONE-SULPHONIC ACID

R—SO_3H diln. (anal.) litres	log *D*	H_2SO_3 *M*/litre (anal.) $\times 10^5$	k' (obsd.) $\times 10^5$	$k_{H_2SO_3}$ $\times 10^5$	k_{R-SO_3H} $(k' - k_{H_2SO_3})$ $\times 10^5$ calc.	Λ_{18} $(k.\phi)$
25	1.398	224.0	1276.0	70	1206	301.5
104	2.017	82.6	328.5	28	301	312
222	2.346	70.0	169.7	24	146	313
421	2.624	25.6	83.0	12	71	299
835	2.922	12.6	41.7	7	35	290

NOTE:— *Values for $k_{H_2SO_3}$ from Hoover and Hunten's data (27).*

System:— Quinone—Sulphurous Acid

Eastman Kodak's product was used. The usual equimolar mixture was prepared and the reaction shown by the following analyses to be one of reduction of quinone to hydroquinone with corresponding oxidation of sulphurous to sulphuric acid; (1) free sulphurous acid, 0.0043 *M*: total acid; (a) with brom

TABLE XV

SPECIFIC CONDUCTIVITY OF CYCLOHEXANONE-SULPHUROUS ACID SYSTEM

R—SO_3H = 219 litres dilution = 88% of total H_2SO_3				R—SO_3H = 222 litres dilution = 88% of total H_2SO_3			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
T °C.	$k.10^5$	T °C.	$k.10^5$	T °C.	$k.10^5$	T °C.	$k.10^5$
18	168.6	145	123.4	18	169.7	125	146.7
45.7	217.9	135	135.9	30	197.8	110	165.0
65	212.1	125	148.2	45	217.8	100	177.9
85	192.3	110	166.9	55	217.8	85	192.7
100	177.4	100	177.2	65	211.5	65	212.5
110	166.2	85	192.7	85	192.7	54	217.8
125	148.2	65	220.0	100	177.9	44.5	213.9
135	135.1	46	207.9	110	164.2	29.5	194.8
145	123.4			125	146.7	18	167.1

NOTE:— *Moles ketone: moles H_2SO_3 = 2.5:1.*

cresol green as indicator and calculating as the bisulphite end-point gave 0.1629 *M*—indicating other and much stronger acids than sulphurous to be present; (b) with phenolphthalein indicator, and calculating to the sulphate end-point gave 0.0829 *M*. Analysis of reaction mixture for sulphates: $BaSO_4$ per 25 cc. sample; 0.4274, 0.4258 gm., av., 0.4766: molarity of $BaSO_4$, 0.0820 *M*, *i.e.*, a check for the result for total acidity.

System:—Resorcinol—Sulphurous Acid

The usual molar mixture analyzed:—sulphurous acid: total, 0.1303 *M*; free, 0.1303 *M*.

TABLE XVI

SPECIFIC CONDUCTIVITY OF RESORCINOL-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling			
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	137.9	100	169.8	145	117.7	63	192.4
30	159.6	110	159.0	135	128.1	45	172.8
47.2	182.0	125	141.0	125	141.0	31.2	160.2
64	194.1	135	129.8	110	159.0	18	137.9
74.5	192.2	145	117.7	100	168.0		
85	182.0			85	182.0		

NOTE:— *No sulphonic acid originally present; free sulphurous acid, 149 litres dilution.*

System:— Phloroglucinol—Sulphurous Acid

Kahlbaum's product "for analysis" was used. Some reaction takes place in equimolar mixture but the sulphonic acid formed is the most unstable yet encountered. It is decomposed during iodometric titration even when ice is used in an attempt to freeze the equilibrium. By varying the conditions of temperature, excess iodine, and speed of operation a maximum (for checked

results) of 7% of the total sulphurous acid (0.0940 *M*) was indicated as combined. In view of the instability of the reaction product this should represent a minimum for the true combination.

TABLE XVII

SPECIFIC CONDUCTIVITY OF PHLOROGLUCINOL-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling			
Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$
18	135.4	100	161.8	145	111.8	65	180.7
30	155.8	110	151.2	135	112.2	43	172.1
44.8	172.1	125	134.5	125	134.0	30	155.8
64.4	179.4	135	122.7	110	151.2	18	135.4
74.5	177.3	145	111.8	100	162.2		
85	172.7			85	173.7		

NOTE:— *Total sulphurous acid, 154 litres dilution.*

System:— Cinnamyl Alcohol—Sulphurous Acid

Kahlbaum's cinnamyl alcohol was recrystallized from ether-ligroin; colorless needles, m.p. 32-33° C. In equimolar mixture with sulphurous acid only a small amount went into solution. After two days:—sulphurous acid: total, 0.0890 *M*; free, 0.0892 *M*, indicating no combination.

TABLE XVIII

SPECIFIC CONDUCTIVITY OF CINNAMYL ALCOHOL-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling			
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$
18	138.0	85	174.5	145	112.2	46	173.8
30	157.0	100	162.6	135	125.0	29.7	153.9
46	173.8	110	152.7	125	137.0	18	135.0
55.5	178.7	125	135.0	110	154.5		
65	180.2	135	123.8	85	174.5		
75	179.5	145	112.2	64	181.2		

NOTE:— *No sulphonic acid originally present; free sulphurous acid, 205 litres dilution. Due to the slight solubility of the alcohol considerable excess sulphurous acid was present.*

Two grams of the cinnamyl alcohol was heated in a sealed tube with 50 cc. of approximately 0.1 *M* sulphurous acid for 1 hr. at 145° C. After cooling the aqueous layer analyzed:— sulphurous acid: total, 0.0770 *M*; free, 0.0711 *M*: sulphonic acid, 0.0059 (7.9% combined). Another sample heated with more dilute acid for 6 hr. at 145° C. on cooling: —sulphurous acid: total, 0.0505 *M*; free, 0.0428 *M*: sulphonic acid, 0.0077 *M* (15% combined).

System:— Tetrahydrobenzene—Sulphurous Acid

Eastman's cyclohexene (5 cc.) was heated in a sealed tube with 50 cc. sulphurous acid for two hours at 145° C. After cooling the aqueous layer:—

sulphurous acid: total, 0.0895 *M*; free, 0.0634 *M*: sulphonic acid, 0.0261 *M* (29.2% combined).

Similar mixtures were heated for 1 hr. at 145° C., cooled, emptied into a nitrogen-filled flask and some of the excess sulphur dioxide removed in vacuum. The liquid analyzed:— sulphurous acid: total, 0.0418 *M*; free, 0.0157 *M*: sulphonic acid, 0.0261 *M* (62.4% combined).

TABLE XIX

SPECIFIC CONDUCTIVITY OF TETRAHYDROBENZENE-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling	
Temp. °C.	$k.10^5$ observed	$k_{H_2SO_3}$ $\times 10^5$ (Hoover & Hunten)	k_{R-SO_3H} $\times 10^5$ calc.	Temp. °C.	$k.10^5$ observed
18	223.3	95.8	127.5	145	282.0
30	258.5	—	—	135	287.5
42	286.2	127.5	158.7	125	292.0
65	315.1	136.4	173.7	110	298.5
85	311.3	136.7	174.6	100	306.5
100	305.0	129.3	175.7	85	311.3
110	300.0	—	—	67	311.3
125	292.0	112.0	180.6	44	286.2
135	286.2	103.9	182.3	30	255.2
145	282.0	94.4	187.6	18	221.9

NOTE:— *Free sulphurous acid originally in cell liquor, 0.00306 M/litre. Values for* $k_{H_2SO_3}$ *by interpolation between Hoover and Hunten's data at 0.00351 and 0.00276 M/litre.*

TABLE XX

HYDROLYSIS OF CYCLOHEXANE SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. *N*/10 I_2 required	H_2SO_3 *M*/litre	R—SO_3H hydrolyzed	
			M/litre	%
60	3.22	0.0161	0.0004	0%
120	3.01	0.0151	none	within
240	3.14	0.0157	none	exptl. error

NOTE:— *Sulphurous acid: total, 0.0418M; free, 0.0157 M; sulphonic acid, 0.0261. Volume of reaction mixture, 10 cc.*

System:—Furfural—Sulphurous Acid

"Refined" furfural from the Miner Laboratories, Chicago, was distilled under reduced pressure and redistilled immediately before being used, b.p. 55-56° C./17 mm.

The equimolar mixture gave:— sulphurous acid: total 0.0855 *M*; free 0.0103 *M*: sulphonic acid, 0.0752 *M*.

TABLE XXI

SPECIFIC CONDUCTIVITY OF FURFURAL-SULPHUROUS ACID SYSTEMS

$\frac{\text{mols R—CHO}}{\text{mols } H_2SO_3}=\frac{1}{1}$ R—SO_3H = 227 litres diln. = 76.5% of total H_2SO_3				$\frac{\text{mols R—CHO}}{\text{mols } H_2SO_3}=\frac{1}{1.8}$ R—SO_3H = 187 litres diln. = 53.5% of total H_2SO_3			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$
18	188.5	145	129.2	18	300	145	178
30	218.5	135	142.1	44.5	367	135	195
42	239.0	125	157.8	65	348	125	218
56	241.5	110	179.8	85	310	110	253
65	235.0	80.5	219.8	100	273	100	276
75	224.3	65	235.0	110	250	85	312
85	214.1	45	235.8	125	217	65	348
100	193.3	30	211.8	135	196	48.5	351
110	179.5	18	183.9	145	178	30	330
125	157.8					18	291
135	142.1						
145	129.2						

TABLE XXII

HYDROLYSIS OF FURFURAL SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	16.22	0.0811	0.0710	94
10 min.	17.17	0.0859	0.0757	100

NOTE:— *Sulphurous acid: total, 0.0855 M; free, 0.0101 M; sulphonic acid, 0.0754 M.*

System:— Furfuryl Alcohol—Sulphurous Acid

Eastman's product was used. An equimolar mixture polymerized very readily, as shown by the development of a chocolate color about 48 hr. after mixing (nitrogen atmosphere). When samples were allowed to stand exposed to the air they turned dark brown in about half an hour. The progress of the reaction with sulphurous acid is shown by the analyses two hours after mixing: sulphurous acid: total, 0.0885 M; free 0.0881 M: sulphonic acid, 0.0004 M. Approx. 20 hr. after mixing:— sulphurous acid: total, 0.0840 M; free, 0.0743 M: sulphonic acid, 0.0097 M (11.5% combined). Two days after mixing:— sulphurous acid: total, 0.0795 M; free, 0.0604 M: sulphonic acid, 0.0191 M (24% combined).

Conductivity-temperature runs were made on each of two lots of reaction mixture the day after their preparation.

A quantity of the reaction mixture was heated in sealed tubes for one hour at 110 ° C. The resulting liquor was colored brown. On analysis it showed:—

sulphurous acid: total, 0.0825 *M*; free, 0.0247 *M*: sulphonic acid, 0.0678 *M* (82.3% combined).

TABLE XXIII

SPECIFIC CONDUCTIVITY OF FURFURYL ALCOHOL-SULPHUROUS ACID SYSTEM

Total H_2SO_3 117 litres dilution				Total H_2SO_3 256 litres dilution			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	226	145	417	18	120.1	145	246.2
30	255	135	405	30	138.4	135	245.2
44.5	276	125	403	45	158.2	125	242.0
54	284	110	403	55	166.8	105	236.5
65	286	100	399	65	169.9	100	234.1
75	298	85	393	75	172.1	85	228.5
86	315	65	377	85	174.4	65	211.9
100	321	39.5	324	100	194.2	47.5	190.3
110	405	30	298	110	228.5	33.6	162.3
125	416	18	251	125	243.5	18	129.9
135	417			135	246.2		
145	417			145	246.2		

TABLE XXIV

HYDROLYSIS OF FURFURYL ALCOHOL SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20°C.

Time, min.	Cc. *N*/10 I_2 required	H_2SO_3 *M*/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	6.45	0.0323	0.0076	11.2
30	6.95	0.0348	0.0101	14.9
60	7.17	0.0359	0.0112	16.5
120	7.24	0.0362	0.0115	16.9
240	7.49	0.0375	0.0128	18.8

NOTE:— *Sulphurous acid: total, 0.0825 M; free, 0.0247 M; sulphonic acid, 0.0678 M.*

System:— Glucal—Sulphurous Acid

The product used was a carefully purified crystalline glucal. Reaction in the equimolar solution took place slowly as shown by the analyses. Two days after mixing:— sulphurous acid: total, 0.0890 *M*; free, 0.0659 *M*: sulphonic acid, 0.0231 *M* (26% combined). Five days after mixing:— sulphurous acid: total, 0.0805 *M*; free, 0.0349 *M*; sulphonic acid, 0.0456 *M*. Nine days after mixing:— sulphurous acid: total, 0.0717 *M*; free, 0.0155 *M*: sulphonic acid, 0.0562 *M*.

A conductivity-temperature run was made of the two-day reaction mixture. The results are given in Table XXV.

After removing excess sulphur dioxide from the five-day reaction mixture the liquid analyzed:— sulphurous acid: total, 0.0625 *M*; free, 0.0154 *M*: sulphonic acid, 0.0471 *M*. The liquid was diluted 10 times and taken through two cycles of temperature change in the conductivity cell. The results are given in Table XXVI.

TABLE XXV

SPECIFIC CONDUCTIVITY OF GLUCAL-SULPHUROUS ACID SYSTEM

First temperature cycle				Second temperature cycle			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^5$
18	146.8	145	202.5	18	150.0	145	226.0
30.5	168.1	135	206.8	44.5	198.5	135	230.0
42	187.7	126	223.5	65	224.3	122	234.2
54	196.1	110	233.0	85	233.0	109	238.5
65	203.9	100	234.2	100	234.2	94.5	237.5
85	200.2	85	234.2	110	233.0	84.5	236.5
100	187.7	67.5	228.6	125	228.6	73	234.2
110	190.2	49.5	208.3	135	230.0		
125	197.2	30	184.9	145	226.0		
135	202.5	18	150.0				
145	202.5						

NOTE:— *Total sulphurous acid = 204 litres dilution = 24% of total sulphurous acid combined in the original reaction mixture.*

TABLE XXVI

SPECIFIC CONDUCTIVITY OF GLUCAL-SULPHUROUS ACID SYSTEM

First temperature cycle				Second temperature cycle			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^6$
18	192.2	145	290.5	18	190.1	145	326.0
30	224.2	134.8	302.1	47	262.1	135	326.0
42	255.2	125	312.5	65	295.0	125	326.0
65	294.0	110	316.5	85	313.8	110	329.0
85	284.0	83	315.0	100	319.0	79	315.0
100	248.0	64.8	295.0	110	323.0	64.5	297.5
110	253.5	45	262.1	125	323.0	45	258.6
125	282.0	30	224.2	135	326.0		
135	286.0	18	190.1	145	326.0		
145	290.5						

NOTE:— *Total sulphurous acid, 160 litres dilution. R—SO_3H, 212 litres dilution.*

The rate of hydrolysis with barium hydroxide of the five-day reaction mixture was determined with the results shown in Table XXVII.

A sample of the reaction mixture heated in a sealed tube at 125° C. for 3 hr. gave a brown liquid analyzing:— sulphurous acid: total, 0.0889 M; free, 0.0118 M: sulphonic acid, 0.0771 M (86.9% combined).

TABLE XXVII

HYDROLYSIS OF THE GLUCAL-SULPHONIC ACID REACTION MIXTURE WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	11.54	0.0577	0.0228	50.0
60	11.49	0.0575	0.0226	49.6
120	11.54	0.0577	0.0228	50.0
240	11.54	0.0577	0.0228	50.0

NOTE:— *Sulphurous acid: total, 0.0805 M; free, 0.0349 M: sulphonic acid, 0.0456 M.*

TABLE XXVIII

HYDROLYSIS OF A PREVIOUSLY HEATED SAMPLE OF THE GLUCAL-SULPHONIC ACID REACTION MIXTURE WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	2.92	0.0146	0.0028	3.6
210	2.83	0.0142	0.0024	3.1

NOTE:— *Sulphurous acid: total, 0.0889 M; free, 0.0118 M: sulphonic acid, 0.0771 M. Volume of samples used, 10 cc.*

TABLE XXIX

VARIATION OF SPECIFIC CONDUCTIVITY OF GLUCAL—SULPHUROUS ACID AT 125° C.

Time hr.	Time min.	$k.10^5$	$(k-k_0)10^5$	Time hr.	Time min.	$k.10^5$	$(k-k_0)10^5$
0	0	194.4	—	1	0	227.2	+32.8
0	2	183.9	−10.5	1	16	229.3	34.9
0	4.5	186.8	− 7.6	1	30	233.0	38.6
0	10	200.3	+ 5.9	1	45	234.2	39.8
0	14.5	208.2	13.8	2	0	237.3	42.9
0	20	212.3	17.9	2	30	240.2	45.8
0	25	215.5	21.1	3	30	247.5	53.1
0	30	218.0	23.6	4	0	250.0	56.6
0	40	221.9	27.5	5	0	255.5	62.1
0	50	224.0	29.6				

NOTE:— *Total sulphurous acid, 203 litres dilution; sulphonic acid, 334 litres dilution. Conductivity cell heated rapidly to final temperature.*

System:— Quercetin—Sulphurous Acid

Eastman Kodak's product was used. Owing to the high molecular weight of quercetin and its limited solubility, a lower concentration of sulphurous acid than usual was used in the equimolar reaction mixture. The reaction proceeded very slowly and the equilibrium was far on the side of the original reactants, as shown by the analyses. Five days after mixing:— sulphurous acid: total, 0.0520 M; free, 0.0437 M: sulphonic acid, 0.0083 M (15.9% combined). Ten days after mixing:— sulphurous acid: total, 0.0513M; free, 0.0399 M: sulphonic acid, 0.0114 M (22.2% combined). Twelve days after

mixing (after removal of excess sulphur dioxide under reduced pressure): sulphurous acid: total, 0.0145 M; free, 0.0090 M: sulphonic acid, 0.0055M (33% combined). Since nearly half the sulphonic acid present had been decomposed, the final equilibrium-combined percentage was probably that indicated.

A sample of the liquid was diluted and a conductivity run made over the usual temperature range. The results are given in Table XXXII.

TABLE XXX

VARIATION OF SPECIFIC CONDUCTIVITY OF GLUCAL—SULPHUROUS ACID AT 135° C.

Time hr.	min.	$k.10^5$	$(k-k)_0 10^5$	Time hr.	min.	$k.10^5$	$(k-k_0)10^5$
0	0	173.9	—	1	1	216.7	+42.8
0	2	168.0	− 5.9	1	15	219.2	45.3
0	4	178.6	+ 4.7	1	30	223 4	49.5
0	6.5	184.9	11.0	1	45	226.0	52.1
0	10	190.2	16.3	2	0	228.4	54.5
0	20	201.5	27.6	2	31	234.2	60.3
0	30	206.0	32.1	3	0	242.0	67.9
0	41	209.7	35.8	4	0	250 0	75.9
0	51	213.0	39.1	5	0	257.0	82.9

NOTE:— *Total sulphurous acid, 216 litres dilution; sulphonic acid, 336 litres dilution. Conductivity cell heated rapidly to the final temperature.*

TABLE XXXI

VARIATION OF SPECIFIC CONDUCTIVITY OF GLUCAL—SULPHUROUS ACID AT 135° C.

Time hr.	min.	$k.10^5$	$(k-k_0)10^5$	Time hr.	min.	$k.10^5$	$(k-k_0)10^5$
0	0	186.5	—	0	45	222.0	+35.5
0	2	185.5	− 1.0	1	0	224.5	38.0
0	4	191.8	+ 5.2	1	30	231.5	45.0
0	6	196.4	9.9	2	30	245.2	58.7
0	10.5	202.0	15.5	3	0	248.5	62.0
0	20	208.1	21.6	4	0	255.3	68.8
0	30	213.8	26.3	5	0	260.5	74.0

NOTE:— *Total sulphurous acid, 208 litres dilution; sulphonic acid, 273 litres dilution. The conductivity cell was heated to 100° C., held at that temperature for half an hour and then the cell contents were raised to the final temperature (zero time).*

TABLE XXXII

SPECIFIC CONDUCTIVITY OF QUERCETIN—SULPHUROUS ACID SYSTEM

Temperature rising				Temperature falling			
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	113.8	100	153.8	145	121.2	60.9	157.0
44	146.0	110	148.2	125	137.0	42.2	146.0
54.5	155.0	125	136.9	110	147.0	18	113.0
65.5	158.9	135	128.4	100	153.2		
85	158.1	145	121.2	85	159.6		

NOTE:— *Total sulphurous acid, 276 litres dilution; sulphonic acid, 745 litres dilution.*

TABLE XXXIII

HYDROLYSIS OF QUERCETIN SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litres	%
At once	10.25	0.0513	0.0114	100
10 min.	10.25	0.0513	0.0114	100

NOTE:— *Sulphurous acid: total, 0.0513 M; free, 0.0399 M; sulphonic acid, 0.0114M.*

LIGNOSULPHONIC ACIDS

The first lignosulphonic acids studied were obtained by heating benzene-alcohol extracted spruce meal with sulphurous acid. After cooling, the liquor was filtered and then electrodialyzed in order to remove any sulphuric acid, excess sulphurous acid, and any other lower molecular complexes present. The apparatus employed was that of Pauli which has been described in detail elsewhere (42). Parchment membranes were used, and an auxiliary constant-head arrangement set up for the water supply to the outer compartments so as to limit pressure variations on the membranes. At the start of electrodialysis 110 volts were used and this value subsequently increased to 220 volts.

The liquor was electrodialyzed for 20 hr., after which it contained 0.052 gm. of total solids per cc. (dried in vacuum at 65° C.) and about 2 mg. of ash per cc. The liquor was diluted ten times and measurements made over the usual temperature range in the conductivity cell with results shown in Table XXXIV.

TABLE XXXIV

SPECIFIC CONDUCTIVITY OF LIGNOSULPHONIC ACIDS FROM SPRUCE MEAL AND SULPHUROUS ACID

Temperature rising				Temperature falling			
Temp. °C.	$k.10^6$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	87.5	100	230.0	145	265.0	65	175.3
30	110.0	110	240.3	135	260.5	45	141.6
44	137.0	125	253.5	125	253.5	30	112.0
63.5	173.8	135	260.5	100	230.0	18	87.5
85	212.0	145	265.0	85	211.0		

TABLE XXXV

HYDROLYSIS AT 20° C. WITH BARIUM HYDROXIDE OF LIQUOR FROM SULPHUROUS ACID COOK

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	Increase H_2SO_3 M/litre
At once	4.28	0.0214	0.0084
30	4.90	0.0245	0.0115
60	5.11	0.0256	0.0126
120	5.11	0.0256	0.0126
240	5.04	0.0252	0.0122

NOTE:— *Iodine titration of liquor equivalent to 0.0130 M H_2SO_3. Volume of samples used, 10 cc.*

Samples of waste sulphite liquors were obtained from the Howard Smith Paper Mills and from the Canada Power and Paper Co., and electrodialyzed. Conductivity data were obtained on the electrodialyzed liquor diluted ten times.

TABLE XXXVI

ANALYSES OF SULPHITE LIQUORS

	Howard Smith	Canada Power and Paper
	gm. per cc.	
Original liquors		
Total solids (65° C. in vacuum)	0.0977	0.0852
Ash	0.0108	0.0121
After 60 hr. electrodialysis		
Total solids (65° C. in vacuum)	0.0439	0.0491
Ash	0.0063	0.0096

TABLE XXXVII

SPECIFIC CONDUCTIVITY OF ELECTRODIALYZED WASTE SULPHITE LIQUOR

Liquor from Howard Smith Paper Mills				Liquor from Canada Power and Paper Co.			
Temperature rising		Temperature falling		Temperature rising		Temperature falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	82.4	145	319.5	18	93.0	145	355.8
29.8	108.1	135	315.0	43	150.0	135	347.5
44.5	138.4	125.5	303.9	65	207.3	125	335.0
65	183.9	91	246.5	85	253.8	110	312.2
85	227.5	85	236.0	100	286.1	90	272.0
100	256.7	60	180.4	110	305.0	64.5	213.6
110	275.8	45.5	147.1	125	329.0	47.4	169.8
125	295.2	31	113.8	135	344.1	30	125.0
135	307.2	18	85.5	145	355.8	18	97.3
145	319.5						

The change in iodine titration on treatment with barium hydroxide (calculated as sulphurous acid) was measured in the case of the electrodialyzed Howard Smith waste liquor (Table XXXVIII).

TABLE XXXVIII

ATTEMPTED HYDROLYSIS OF ELECTRODIALYZED WASTE SULPHITE LIQUOR WITH BARIUM HYDROXIDE

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	Increase H_2SO_3 M/litre
At once	1.43	0.0072	zero
30	1.53	0.0077	within
60	1.27	0.0064	exptl.
120	1.62	0.0081	error
240	1.57	0.0079	

NOTE:— *Iodometric titration equivalent to 0.0078 M* H_2SO_3.

Samples of lignin prepared by the Beckmann-Liesche-Lehmann (1) method were heated in sealed tubes with 3% sodium bisulphite solution in water free from oxygen. The tubes were flushed out with nitrogen before loading and heated at 110° C. for one hour and then at 125° C. for two hours. Some lignin went into solution. After cooling and electrodialyzing to remove excess sulphite the liquor contained: total solids, 0.0076 gm. per cc.; ash, 0.0020 gm. per cc. The liquid was diluted with its own volume of water free from oxygen and the conductivity determined (Table XXXIX).

TABLE XXXIX

Specific conductivity of sulphonic acids from Beckmann-Liesche-Lehmann lignin

Temperature rising				Temperature falling			
Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$
18	181	100	506	145	537	88.5	436
30	234	110	536	135	536	65	339
43	288	125	548	122	521	48	270
65.8	387	135	544	110	496	31	224
85.5	464	145	537	100	466	18	171

Summary and Conclusions

1. The sulphonic acids of nuclear aldehydes are typically much more unstable than those with $—SO_3H$ joined to aliphatically bound—CHO groups.

2. A quantitative method has been developed for following the equilibrium shift in solutions of these unstable sulphonic acids on dilution, and used to study their relative stability.

3. The reaction of sulphurous acid with compounds containing a latent aldehyde group such as glucose is characterized by a very slow velocity. A relation is indicated between the system glucose-sulphurous acid and the cyclic-open chain glucose equilibrium in aqueous solution.

4. The sulphonic acids of saturated ketones are very unstable. They are characterized by very ready decomposition and an equilibrium far on the side of the original reactants.

5. A method, based on some previous observations of Hoover and Hunten, for quantitatively measuring the rate of hydrolysis of the sulphonic acids with barium hydroxide, has been developed. The mechanism of this hydrolysis is a displacement of the aldehyde-sulphurous acid-sulphonic acid equilibrium. Its rate is a characteristic property of each type of sulphonic acid studied and has been used as a guide to the constitution of the sulphonic acids.

6. Unsaturated ketones form the —C=C— type sulphonic acids at room temperature. A mechanism for this reaction is proposed.

7. Compounds containing phenyl groups attached to a $—CO—CH_2—$ linkage do not react with sulphurous acid over the temperature range 18-145° C.

8. Cyclic ketones react similarly to aliphatic ketones and give sulphonic acids and not sulphite esters.

9. For the system quinone-sulphurous acid the rapid oxidation-reduction reaction completely inhibits sulphonic acid formation.

10. Resorcinol does not react in its tautomeric alicyclic form with sulphurous acid over the temperature range 18-145° C.

11. A slight reaction takes place between phloroglucinol and sulphurous acid, but the sulphonic acid formed is extremely unstable.

12. The reaction of sulphurous acid with compounds in which an ethylene linkage is the only reactive group takes place only very slowly and at high temperatures. Confirmatory evidence is thus provided for the previously proposed mechanism of formation of —C=C— type sulphonic acids.

13. The reaction of furfural with sulphurous acid is that of a normal nuclear aldehyde.

14. Study of the system furfuryl alcohol-sulphurous acid shows that the sulphonic acid group enters the furane ring. This action takes place slowly at room temperature and very rapidly when the reaction mixture is heated. The preliminary formation of an oxonium addition product is indicated, and a mechanism for the complete reaction scheme for this system is proposed.

15. The interaction of the pyrone derivative, glucal, and sulphurous acid provides strong evidence for the primary formation of an oxonium addition product. At high temperatures the sulphurous acid adds on to the ethylene linkage of the pyrone ring. The rate of formation under critical conditions of this —C=C— type sulphonic acid shows definitely that the oxonium addition product is an intermediate in the formation of the —C=C— sulphonic acid. A mechanism for the reaction is postulated and strong confirmation found for a similar mechanism previously proposed for the reaction of furfuryl alcohol with sulphurous acid. Further confirmatory evidence based on the rate of hydrolysis of the sulphonic acids by barium hydroxide is submitted.

16. The reaction of sulphurous acid with the flavonol, quercetin, shows the same characteristics as that of other ketones. Possible reasons for this on the basis of the previously postulated reaction scheme are suggested.

17. Conductivity-temperature curves for lignosulphonic acids obtained from various sources indicate that the products are sulphonic acids of the —C=C— type.

18. Other evidence points to an oxonium addition product with a ring oxygen being a probable intermediate in the formation of the final and more stable lignosulphonic acid. The relation of such a mechanism to the experimental conditions employed in the commercial process of the manufacture of sulphite pulp is indicated.

19. The formation of lignosulphonic acids through the medium of a reaction involving a phenolic nucleus in its tautomeric form is highly improbable.

20. The fact is emphasized that for the formation of a lignosulphonic acid a carbonyl group does not necessarily have to be present.

21. Strong additional evidence is presented for the presence of a heterocyclic ring containing oxygen, and for an ethylene linkage in the lignin molecule. The latter is indicated to be in the same ring system as the oxygen atom.

Acknowledgments

The authors wish to express their thanks to Mr. J. Barsha for his preparation of crystalline glucal, and to Mr. A. F. W. Cole for supplying samples of Beckmann-Liesche-Lehmann lignin.

References

1. BECKMANN, E., LIESCHE, O. and LEHMANN, F. Z. angew. Chem. 34: 285-288. 1921.
2. CAMPBELL, W. B. J. Am. Chem. Soc. 51: 2419-2420. 1929.
3. CROSS, C. F. and BEVAN, E. J. Researches on Cellulose. III. (1905-1910) Longmans, Green. 1912.
4. FREUDENBERG, F., HARDER, M. and MARKERT, L. Ber. 61: 1760-1765. 1928.
5. FREUDENBERG, K., ZOCHER, H. and DÜRR, W. Ber. 62: 1814-1823. 1929.
6. FRIEDRICH, A. Z. physiol. Chem. 168: 50-67. 1927.
7. FRIEDRICH, A. and DIWALD, J. Monatsh. 46: 31-46. 1925.
8. FRIESE, H. Ber. 62: 2538-2550. 1929.
9. FUCHS, W. Ber. 54: 245-249. 1921.
10. FUCHS, W. Ber. 54: 484-490. 1921.
11. FUCHS, W. Ber. 60: 957-962. 1927.
12. FUCHS, W. Ber. 60: 1131-1133. 1927.
13. FUCHS, W. Ber. 60: 776-782. 1927.
14. FUCHS, W. Ber. 60: 1327-1330. 1927.
15. FUCHS, W. and ELSNER, B. Ber. 53: 886-898. 1920.
16. FUCHS, W. and HORN, O. Ber. 61: 2197-2202. 1928.
17. FUCHS, W. and HORN, O. Ber. 62:2647-2653. 1929.
18. GORTNER, R. A. Outlines of biochemistry. Wiley. N.Y. 1929.
19. HÄGGLUND, E., JOHNSON, T. and TRYGG, L. H. Svensk Pappers—Tid. 32: 815-823. 1929.
20. HÄGGLUND, E. and URBAN, H. Cellulosechemie, 8: 69-71. 1927.
21. HAWLEY, L. F. and WISE, L. E. The chemistry of wood. Chem. Cat. Co. N.Y. 1926.
22. HIBBERT, H. and MARION, L. Can. J. Research, 2: 364-375. 1930.
23. HIBBERT, H. and PHILLIPS, J. B. Can. J. Research, 3: 65-69. 1930.
24. HIBBERT, H. and ROWLEY, H. J. Can. J. Research, 2: 357-363. 1930.
25. HIBBERT, H. and SANKEY, C. A. Can. J. Research, 4: 110-118. 1931.
26. HIBBERT, H. and TAYLOR, K. A. Can. J. Research, 4: 240-253. 1931.
27. HOOVER, G. I., HUNTEN, K. W. and SANKEY, C. A. J. Phys. Chem. 34: 1361-1386. 1930.
28. JONAS, K. G. Papier-Fabr. 26: 221-229. 1928.
29. KERP, W. Arb. kais. Gesundh. 21: 141-155. 1904.
30. KLASON, P. Ber. 53: 706-711. 1920.
31. KLASON, P. Ber. 53: 1864-1873. 1920.
32. KLASON, P. Ber. 55: 448-455. 1922.
33. KLASON, P. Ber. 56: 300-308. 1923.
34. KLASON, P. Ber. 58: 1761-1764. 1925.
35. KLASON, P. Ber. 61: 171-176. 1928.
36. KLASON, P. Svensk. Kem. Tid. 29: 5-16; 47-52. 1917.
37. KÖNIG, F. Cellulosechemie, 2: 93-101; 105-113; 117-122. 1921.
38. KÜRSCHNER, K. Zur Chemie der Ligninkörper. Ferdinand Enke, Stuttgart. 1925.
39. LABBÉ, H. Bull. soc. chim. 21: 1077-1080. 1899.
40. MELANDER, K. H. A. Chem. Abstr. 16: 4342. 1922.
41. MOUREU, C., DUFRAISSE, C. and JOHNSON, J. R. Ann. chim. 7: 5-13. 1927.
42. PAULI, W. Biochem. Z. 152: 355-359. 1924.
43. PAULY, H. and FEUERSTEIN, K. Ber. 62: 297-311. 1929.
44. PREGL, F. Quantitative organic microanalysis, tr. by Fyleman. London, 1924.
45. PUMMERER, R. and GUMP, W. Ber. 56: 999-1008. 1923.
46. RASSOW, B. and ZIÇKMANN, P. J. prakt. Chem. 123: 189-234. 1929.

47. Samec, M. and Rebek, M. Kolloidchem. Beihefte. 19: 106-114. 1924.
48. Schrauth, W. Z. angew. Chem. 36: 149-152. 1923.
49. Stelling, O. Cellulosechemie, 9: 100-102. 1928.
50. Williams, D. M. and James, T. C. J. Chem. Soc. 343-347. 1928.

THE DISCONTINUITY IN THE VELOCITY COEFFICIENT OF A CHEMICAL REACTION AT THE CRITICAL TEMPERATURE[1]

By H. S. Sutherland[2] and O. Maass[3]

Abstract

An account is given of an hypothesis dealing with the mechanism of chemical reactions. This hypothesis was the *raison d'être* for the experimental work, which consisted in measuring the rate of a chemical reaction in a system over a temperature range including the critical temperature and under conditions such that there was a continuity of concentration, when passing from the liquid to the gaseous state of aggregation.

An experimental technique for the investigation of reaction mixtures under high pressures and at relatively high temperatures was developed. This method includes several new features which should find considerable application in investigations of this kind.

In the reaction investigated, *viz.*, that between propylene and hydrogen chloride, it was conclusively shown that the velocity of the reaction increases with rise in temperature in the liquid state, and that above the critical temperature the velocity of the reaction becomes practically zero. A new hypothesis was put forward with the object of suggesting further work. It depends on regional orientation of molecules in the liquid state which undergoes a rapid diminution at the critical temperature.

The work described in this paper was undertaken as a result of a definite hypothesis gradually developed in this laboratory during the last ten years, in connection with investigations of the reactions between halogen hydrides and unsaturated hydrocarbons. Briefly, this hypothesis is, that in certain reactions, the temperature coefficient is governed not solely by activation phenomena, but also by orientation of molecules, with the result that in these reactions it might actually have a negative value in a certain temperature range. With this in view, the investigation of the reaction between hydrogen chloride and propylene was undertaken over a temperature range, which extended well above the critical temperature, and under pressure conditions such that at high temperatures the density of the reaction mixture could be made to correspond to that prevailing in the liquid at the lower temperatures. Therefore, a brief review of previous work will be given, followed by a detailed account of the experimental data required.

The addition reactions between acetylene, allylene, ethylene, propylene, α-butylene, β-butylene, γ-butylene and the halogen hydrides in the liquid state were examined (4-12, 14) and it was shown that they fall into two classes. Acetylene and ethylene were found to be non-reactive, whereas the others reacted homogeneously and at a rate that could be reproduced with considerable accuracy. A striking experiment in this connection may be quoted (11, 12). A liquid mixture of 25 mole per cent propylene, 25% ethylene and 50% hydrogen bromide, when allowed to stand at room temperature in a sealed

[1] *Manuscript received June 22, 1931.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada.

[2] *Postgraduate student at McGill University and holder of a studentship under the National Research Council of Canada.*

[3] *Professor of Physical Chemistry, McGill University.*

tube, was found on analysis to have undergone a reaction in which the propylene alone took part, while the ethylene and a corresponding amount of hydrogen bromide remained uncombined.

The question therefore arose early in these investigations as to the difference between ethylene and acetylene, and the other unsaturated hydrocarbons. It was thought that with the first two a decidedly smaller molecular attraction for the halogen hydride might account for their inertness. To test this, freezing-point curves were determined where experimentally possible, and it was indicated that whereas acetylene and ethylene do not form molecular compounds, all the others do. Consequently the first stage of the hypothesis mentioned in the opening paragraph may be summarized as follows. If a reaction is possible between *A* and *B*, the rate of this reaction is greatly increased if there is a molecular attraction between them, especially if this attraction is sufficiently great to induce molecular complex formation between *A* and *B*. In other words, the velocity of the reaction in the liquid state is influenced by the magnitude of the molecular attraction existing between the reactants. When two molecules, one of *A* and one of *B*, have a marked attraction for one another as molecules, it seems reasonable to suppose that there are chance approaches, where they will remain for a longer time in contact than if no such attraction existed. Furthermore this would be accompanied by an orientation conducive to interatomic change in the case when relatively large molecular attraction occurs. Where such a molecular attraction does not exist, a catalyst is necessary, and probably it functions in a manner to provide the equivalence of the molecular attraction in bringing the two together.

It is of course necessary for the reacting pair to have the proper activation in order to make the interatomic change possible. From this point of view a rise in temperature will increase the number of reacting molecules, *i.e.*, the rate of reaction, but on the other hand it will tend to diminish the number of properly orientated molecules, as well as the time of close approach due to increased thermal agitation. This will have a tendency to decrease the number of reacting molecules. This applies only to a system in which there exists a particularly pronounced attraction between the reacting pair. It is conceivable therefore that actually a negative temperature coefficient is possible in the case of a reaction where orientation and a tendency towards complex formation play a large part.

A number of experiments appeared to be in agreement with the views put forward above. Concentration of the reaction mixture should play an exceptional part in the rates of reaction. It was found that when propylene and hydrogen chloride in the gaseous state were mixed at atmospheric pressure, no reaction took place even after a year, although on the basis of concentration, with regard to any order of reaction, an easily detectable amount of combination should have occurred. Experiments followed in which the pressure of the gaseous reaction mixture was increased (9, 10). At higher pressures inconsistent results were obtained, due to the experimental difficulty in mixing the

constituents without at any time having a liquid phase appear. The moment a liquid phase appeared, reaction took place.

It is now possible to state more clearly the experimental problem which confronted the present writers. To carry out a thorough investigation, taking the reaction between propylene and hydrogen chloride as the most convenient to examine, a technique had to be devised to make possible the mixing of these substances in any desired proportions in the gaseous state. The mixture had then to be brought to any desired pressure above the critical temperature without at any time having the liquid phase appear. Furthermore it had to be possible to investigate the reaction temperature coefficient of the mixture at any temperature where the liquid might exist. To do this it was also necessary to determine the critical temperatures of the mixtures examined as well as the density of such mixtures, if any quantitative conclusions were to be drawn. The apparatus described has answered all of the above requirements and involves a number of new features which should prove useful in a large number of investigations carried out at relatively high pressures and temperatures.

Before proceeding to a description of the experimental work, a few more details concerning the propylene-hydrogen chloride reaction must be recapitulated. First, it has been definitely proved that the reaction in the liquid state is homogeneous and not influenced by the materials of the containers or by a mercury surface. Two separate investigations of this point will be published shortly. The product of the reaction between propylene and hydrogen chloride is mainly isopropyl chloride. A secondary reaction, which diminishes in amount with rise in temperature, also takes place (9, 10, 14). The only influence that this had in the present investigation was a slight correction which had to be made in connection with the analysis of the reaction at some of the relatively lower temperatures. It was shown that the velocity of the reaction greatly increases with the amount of hydrogen chloride present (9, 10). Consequently a mixture of two molecular volumes of hydrogen chloride to one of propylene was used throughout, because the rates of reaction of this mixture proved to be most convenient from the point of view of the time for an easily measurable amount of reaction to occur.

Experimental

Preparation of Reactants

The propylene was prepared by the dehydration of isopropyl alcohol over alumina at 360° C., and purified by low temperature fractionation, as described elsewhere (4, 5, 11, 12). The hydrogen chloride, prepared in the usual manner, was dried with phosphorus pentoxide and condensed with solid carbon dioxide in vacuum. It was redistilled and the middle fraction was stored for use.

Apparatus

The apparatus which had to answer the requirements mentioned in the introduction, passed through various stages of development, and several years of preliminary investigation were required. The final form, as developed

during the last year, in which all the difficulties were overcome, is the only one described. The early development was carried out by C. C. Coffin (5).

The reaction mixture, whether in the gaseous state during mixing, or later on when compressed to high pressure, was allowed to come into contact only with glass which had been carefully cleaned and mercury which had been subjected to careful purification and distillation. Moisture was eliminated by heating the apparatus while completely evacuated. The apparatus as

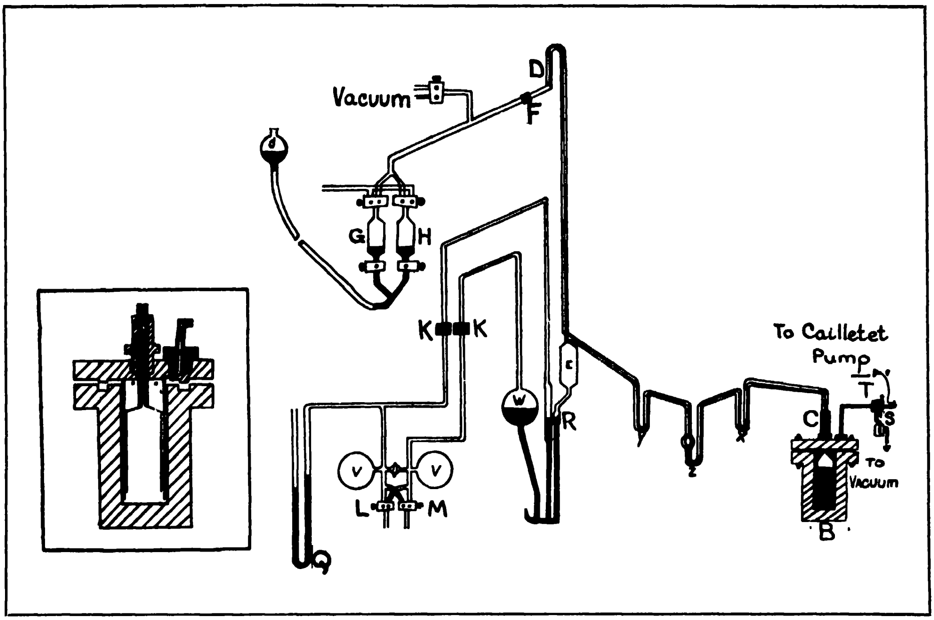


FIG. 1. *Diagrammatic representation of filling apparatus, reaction bulb and pressure apparatus.*

finally developed is shown diagrammatically in Fig. 1. A heavy thick-walled bomb, *B*, turned out of shafting steel was provided with a cover having two inlets. Into one of these, exactly in the centre of the cover, was threaded a large packing nut bearing a steel tube, *C*, which formed the sleeve of a metal joint fastened to the outside of the glass capillary. This will be described later. The glass tube, about 8 mm. Pyrex capillary, was passed through this sleeve and connected with a glass bell within the bomb, as shown in the figure. The capacity of the bell was about 100 cc., while the total capacity of the bomb was about half a litre. Into the inside of the bomb cover was threaded a piece of 2-in. iron pipe, as shown in the insert of the diagram. This was just larger than the glass bell, and served to protect it and eliminate any possible side sway, thus preventing the rigid capillary from breaking at the point where it joined the bell. In early experiments this was a major difficulty.

The bomb was about half filled with clean mercury, and the cover carrying the glass bell, capillary, metal joint, etc., was fastened on with six ½-in. bolts. The bomb was then filled with oil (glycoline) through the other inlet, which

was connected by means of a steel capillary tube to a Cailletet oil pump and a pressure gauge. By pumping oil into the bomb the mercury could be forced up into the bell and act as a piston to compress the gases within it. In view of the relatively low critical pressures of the gases used, this method was preferred to the use of compressed gas for obtaining high pressures. Likewise the explosion risk was practically reduced to zero.

The Pyrex capillary connecting the bell-shaped compression chamber with the reaction bulb, Z, was bent in the form of a U, as was that connecting Z with the measuring bulb, E. The gas mixture was compressed by the Cailletet pump. This was a very good method for obtaining the high pressures but was useless for maintaining them for any appreciable time without continual pumping, owing to leaks in valves, etc. Hence a method was devised which gave the equivalence of an inexpensive absolutely leak-proof valve. Mercury could be frozen in Y while the gas mixture was in the compression chamber. The gases could then be compressed into the reaction bulb and mercury from the bomb forced over into the U-tube, X, where it was frozen. Thus the compressed gases could be kept under any conditions for any desired periods of time without the slightest possibility of either a leak or volume change. The remainder of the apparatus was used for mixing and measuring the reagents and will be described, along with the procedure for making a run.

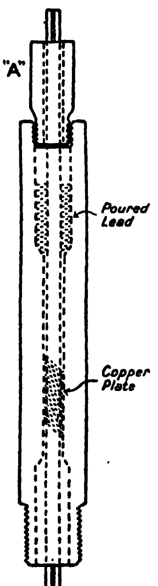


FIG. 2. *Metal sleeve attached to glass capillary with oil in pressure bomb.*

In the face of the flanged end of the bomb was a circular slot to hold a lead gasket, into which a corresponding ridge in the cover could be drawn by tightening the bolts. This gasket was protected from mercury by a layer of heatproof paint. Other gaskets were made of a phenol-resin-linen composition, such as is used for electrical insulation.

Metal Joint Attached to the Outside of the Glass Capillary

Essentially the problem called for a joint which would withstand more than 100 atm. pressure at 150° C. In general two main types of joints were investigated. First, those which would withstand oil pressure, and second, those which would hold mercury. The latter were the more difficult to make.

Fig. 2 shows the sweated type of joint. It consists of a steel sleeve about six inches long with a bore of 12 mm. The tube is constricted as shown in the diagram to about 8 mm.,—slightly larger than the size of the glass capillary tube. The cap, A, screws into the top of the sleeve, as shown, and has an opening just large enough for the capillary.

A three-foot length of Pyrex capillary was alternately swelled and constricted slightly about six inches from one end. This "corrugated" part of the tube was painted with a mixture of gold chloride in oil of lavender and allowed to dry at 100° C. By gradual heating, first in a smoky flame and then in a blast lamp, the oil was burned away and a thin film of conducting gold was fused into the glass. This film

was then electroplated about 1 mm. thick with copper and sweated into the constricted steel sleeve. The top part of the sleeve was filled with molten lead and the cap screwed on. The constrictions and swellings in the capillary prevented it from being forced out of the metal sheath by the pressure. The cap at the top stopped any side sway of the capillary and prevented it from snapping off at the top of the lead. In early types of joints the rigidity at this point caused such a difficulty.

A joint of this type is excellent for withstanding oil or gas pressure. Of course in contact with mercury the lead and copper parts were quickly attacked. Attempts were made to use various methods to protect them. Heat-resisting paints and high melting resins were poured in the bottom part of the metallic sleeve. These were used fairly successfully at room temperatures, but were non-permanent and permeable above 100° C.

Fig. 3 shows another type of joint, designed to withstand pressure when in contact with mercury. Its main advantage is its simplicity and the ease with which it may be taken apart. It consists of a flanged steel tube with rubber gasket and cover to fit. The tube is 3 in. long with a bore of 8 mm., and has a 2½-in. flange at one end. The steel cover, which contains a steel and a rubber disc, is fastened to the flange by four bolts as shown in the diagram. It is so arranged, that by tightening four bolts in the top of the cover any desired pressure can be exerted on the steel disc and thence on the rubber gasket. The cover and both discs have an 8-mm. hole in the centre. The Pyrex capillary was run through the tube, rubber gasket and steel disc. The cover was then bolted on, and the bolts in the top of the cover, X, were tightened. This forced the steel disc against the rubber and produced enough pressure to make an absolutely tight joint between the glass and the gasket. Great care had to be taken to tighten all the bolts equally, so that no shearing could take place. The rubber was extruded somewhat along the capillary tube in both directions and tended actually to pull the glass apart. With capillary tubing below 6 mm., this presented a difficulty, but no trouble was experienced with the larger tubing or a tubing bulbed within the rubber gasket. This is the only type of joint with which any practical success was achieved in withstanding mercury pressures at temperatures above 50° C.

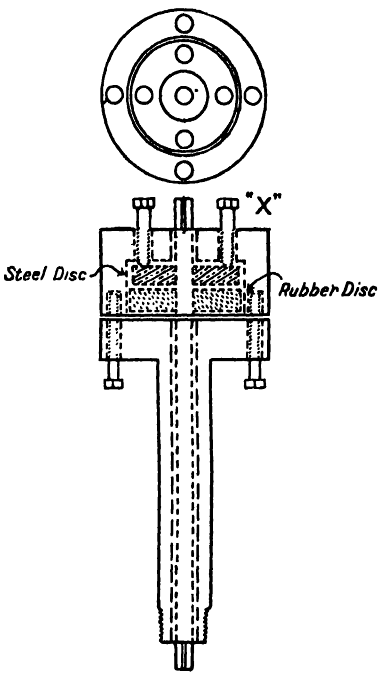


FIG. 3. *Metal sleeve attached to glass capillary with mercury in pressure bomb.*

Both types of joints were used in this investigation. In the earlier experiments a joint of the first type was used, it being only necessary to withstand

oil pressures. Later the apparatus was changed somewhat and the second type of joint was employed. This change consisted of merely inserting another bomb containing mercury between the oil pump and the bomb, *B*, Fig. 1. Thus only mercury was pumped into *B*. The advantages of this system are twofold. First, it eliminates any possibility of oil creeping around the bottom of the bell during manipulation, and second, it is not necessary to redistil the mercury when the bomb, *B*, is taken apart.

Glass Reaction Bulbs

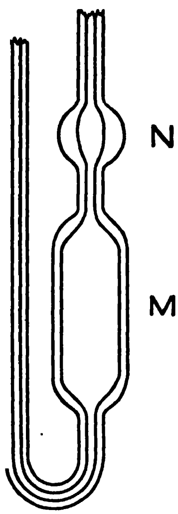


Fig. 4. *Reaction bulb.*

It was soon discovered that, although almost any thick-walled Pyrex bulb would withstand the pressure required, a certain design had a distinct advantage over all others. This advantage lay essentially in the technique of operation. Calculations showed that the reaction bulbs should have a capacity of from 0.2 to 0.3 cc. The disadvantage of such a small bulb was the difficulty in obtaining the correct mercury levels in the capillary tubing. A larger bulb corrected this, but the gases were compressed at one end of the bulb, giving a large mercury surface, an undesirable factor. Fig. 4 shows the type of bulb devised. It combined both the advantages of the small and large bulb. The upper section, *N*, had a capacity of about 0.25 cc., while the lower one held 3 cc. When the mercury to be levelled reached the small bulb, it dropped through to the bottom of the large bulb. The flow was then stopped by freezing the seal, *Y*, Fig. 1. The gases were then compressed and bubbled through this small quantity of mercury into the large bulb, and were finally compressed in the small bulb, *M* being by this time full of mercury. Thus the mercury surface in contact with the gas at high pressures is only twice the area of the capillary tube.

Manipulation of Apparatus

The mercury was first pumped up the capillary tubing, filling the reaction bulb, until it started to drop into *E*, Fig. 1. It was then frozen by surrounding *Y* with a carbon dioxide freezing mixture. The rest of the system was evacuated. The propylene and hydrogen chloride were admitted at known pressures to bulbs *G* and *H* respectively. Thus an equimolecular mixture, 2 : 1 mixture and so on could be obtained. By opening proper taps and raising the mercury reservoir, *J*, the gases were forced over into *E*. When the mercury had reached the mark, *D*, the taps at *G* and *H* were closed. The two mercury systems were thus kept entirely separate, so that no tap grease could be carried over into the measuring bulb, or reaction bulb. The bomb and manometer system of Pyrex were connected with the soft glass apparatus through a graded seal, *F*, and two deKhotinsky joints, *KK*.

By means of the two-way taps, *L*, *M*, pressure or vacuum could be applied to the mercury in the manometer connected to *E*. Thus it could be levelled at the zero mark, *R*, and the actual pressure of the mixed gases in *E* measured

on the open manometer, Q. The pressure and temperature having been measured, the mercury seal, Y, was melted and the gases were forced into the bomb by opening S to vacuum and applying pressure in the reservoir, W. As soon as the mercury started to drop through the reaction bulb the seal, Y, was frozen. Valve S was then closed and T opened, and the oil pump was operated. The gases were compressed slightly and the pressure released to get the small amount of mercury, which was in the bottom of the reaction bulb, back in the bomb. The pressure was then applied to the desired extent and the gases were compressed in the small reaction bulb. The mercury seal, X, was then frozen. With a little experience the amount of gas originally taken could be varied so that the compressed gases would just fill the small reaction bulb. The time taken to bring the gases from E into the small reaction bulb was about one minute. As soon as X was frozen, the oil pressure was released through the pump. At the end of the run the oil pressure was built up to about one third its original value and the seal, X, melted. Great care had to be taken at this point and the melting had to take place from the arms of the U down towards the bend. Otherwise the expansion of the mercury on melting cracked the glass tubing. The pressure was then released and the gases went back into the glass bell in the bomb. The seal, Y, was then melted and the gases were drawn back into E, after which Y was again frozen and the pressure and temperature of the gas read as before. Thus the amount of reaction was determined. The temperature in E could be regulated, in order that the reaction product (isopropyl chloride) would be measured as a gas. A typical example of the calculations involved, where reaction had taken place, illustrates the means of determining the extent of such a reaction.

Reactants measured in G and H (equal volumes): HCl, 250 mm.; propylene, 125 mm. Therefore the molecular proportions were:— propylene: HCl ::1: 2. Initial temperature of E, 27° C. Initial pressure of reactants in $E = 762 - 370 = 392$ mm. The gases were forced into the reaction bulb where they were condensed. Temperature of reaction bulb, 50° C.

After one hour. Temperature of E, 27° C. Pressure of products in E, 366 mm.

$$\% \text{ Reaction} = \frac{\text{(Initial-final)pressure of reactants} \times 100}{\text{Initial partial pressure of propylene}}$$

$$\text{In this case} = \frac{\text{(Initial-final) pressure of reactants} \times 100}{\frac{1}{3}\text{(Initial pressure in } E)}$$

$$= \frac{3 \times (392 - 366) \times 100}{392}$$

$$= 19.8\%.$$

In carrying out true gas reactions the reactants always had to be kept above their critical temperatures. Hence the bomb, B, was heated in a large oil bath and the capillary tubing electrically. The temperature of the reaction bulb was controlled by a smaller transparent oil bath.

The pressures were measured on a hydraulic Bourdon gauge reading up to 3500 lb. per sq. in. This gauge was checked against the vapor pressure of

It is obvious that in discussing these results the density of the reaction mixture is of interest both in the gaseous and liquid states. The methods of determining these densities and the critical temperatures of the mixtures have already been described. The following data were obtained.

TABLE II

CRITICAL TEMPERATURES OF PROPYLENE-HYDROGEN CHLORIDE MIXTURES

Propylene, mole %	100	66.6	50*	33.3	0
Critical temperature, °C.	92.1	84	75	70	52

**Burst the bulb on appearance of less dense liquid phase.*

TABLE III

DENSITIES OF A MIXTURE OF PROPYLENE AND HYDROGEN CHLORIDE (1:2)

	Liquid			Gas					
Temperature, °C.	0	20	50	78	78	78	78	78	78
Pressure, atm.				61	71	74.5	76.5	80.5	102
Density, gm. per cc.	0.80	0.73	0.40	0.16	0.20	0.22	0.24	0.26	0.42

The relation between critical temperature and composition (Table II) is quite typical of a binary mixture in which one component is more volatile than the other. In such a mixture the gaseous phase even considerably above the critical pressure is never absent above the critical temperature of the more volatile compound (in this case, the hydrogen chloride). Hence 52° C. is the highest temperature at which an all liquid phase is possible at pressures up to 100 atm. The 2:1 mixture has a critical temperature of 70° C., so that above this temperature only the gaseous phase can exist. Between 52 and 70° C. for a 2:1 mixture there will therefore always be present a certain amount of gaseous phase, and liquid phase, the relative proportions of which diminish with rise in temperature.

Table III shows that the density of the 2:1 gas mixture above its critical temperature and at a pressure of 100 atm. is greater than the extrapolated density of the liquid mixture for that temperature, and is actually equal to the density of the liquid mixture at 50° C.

A few words to recapitulate what is known up to this stage are now in order. The velocity of reaction of a 2:1 mixture in the liquid state was measured by Sivertz (9, 10) at 0 and 20° C. and was shown to have a decidedly positive temperature coefficient. It is known therefore that up to 20° C. the velocity of the reaction increases, but that at 105° C. it is immeasurably small. Furthermore, that this is not a question of concentration is shown by the density measurements (Table III). When these results were obtained the authors speculated as to the form which the rate of reaction-temperature curve would take. That an inversion takes place somewhere between 20 and 105° C. is obvious.

Two questions therefore arise. First, will a reaction take place at all above the critical temperature? Second, does the temperature coefficient of the velocity of reaction in the liquid state show a point of inversion before the critical temperature is reached? Attempts to answer these two questions will be made in the two subsequent sections.

Experiments were made above the critical temperature and in the range where they were conclusive. The data are shown in Table IV.

TABLE IV
RESULTS OBTAINED IN EXPERIMENTS ABOVE THE CRITICAL TEMPERATURE

Experiment No.	Temperature, °C.	Pressure, atm.	Mixture (HCl: Propylene)	Time, hr.	Result
11	85	100	2:1	2	No reaction
12	80	105	2:1	2	No reaction
13	78	105	2:1	2	No reaction
14	75	100	2:1	2	No reaction

The above data show that within 5° C. of the critical temperature of the mixture no reaction occurs. Experiments at temperatures between 75° C. and the critical temperature give rather indeterminate results. The velocity of reaction in the liquid state follows a normal course. At 72° C., a 2:1 reaction mixture was found to give a reaction, but the curve shows acceleration to take place. Perhaps this is brought out most clearly by a graphic representation, Fig. 5, giving the amount of reaction for a liquid reaction, 50° C., and the reaction just above the critical temperature, 72° C. The interpretation of the forms of these curves is that just above the critical temperature the threshold condition exists in which the reaction is at all possible. The products of the reaction are much less volatile so that once the reaction starts, a condensing medium is formed and the reaction takes on a more normal course. Thus at 72° C. only the first part of the curve shows acceleration. A number of experiments between 75 and 70° C. gave this type of curve. The highest temperature at which a reaction was at all possible was 74° C.

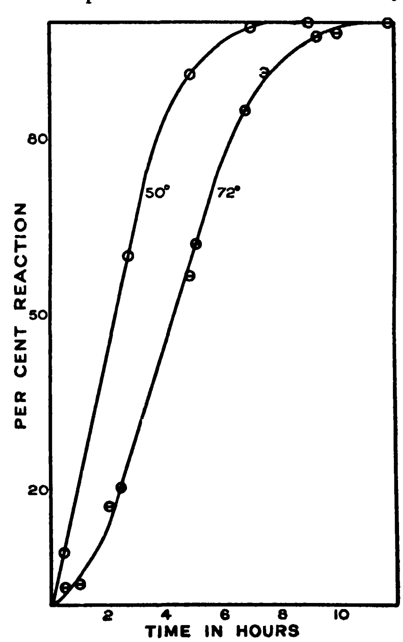


FIG. 5. *Typical reaction curves for reaction below critical temperature of both components and for reaction just above critical temperature of mixture.*

It is therefore shown by these experiments that no reaction takes place to within a few degrees of the critical temperature. Whether the change between

reaction and no reaction is less than a 4° C. interval remains to be investigated. It is certainly not greater.

The measurement of the velocity of reaction in the liquid state can be carried out very readily up to a temperature of 52° C. This was done and the results obtained are shown in Table V.

TABLE V

VELOCITY OF THE REACTION OF PROPYLENE AND HYDROGEN CHLORIDE IN THE LIQUID PHASE

Temperature, °C.	0	20	50
Velocity of reaction, % per min.	0.146	0.208	0.333

It is seen that the velocity of the reaction increases in a regular manner up to 50° C.

The interval between 50 and 70° C. presents an experimental difficulty which cannot be completely overcome with regard to the velocity of the reaction in the liquid phase. Due to the low critical temperature of hydrogen chloride, a gaseous phase is always present*. The procedure followed however in making an estimate was to measure the velocity of the reaction, irrespective of the fact that the gaseous phase was present, at 55 and 69° C. The velocity of the reaction increased from 55 to 69° to exactly the same extent as over a similar range just below 50° C. This would therefore indicate that the velocity of reaction in the liquid continues to increase right up to 69° C. In spite of the presence of the gas phase it is practically equal to that at 50° C.

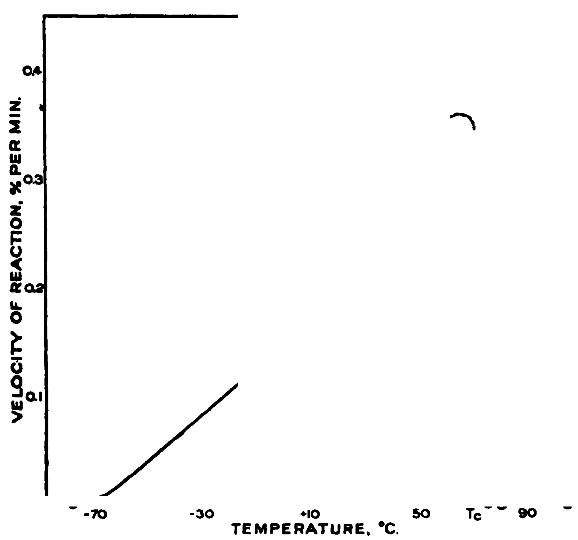


FIG. 6. *Reaction velocity over complete temperature range.*

It follows therefore that the velocity of the reaction in the liquid phase increases with rise in temperature until the near neighborhood of the critical temperature is reached. Perhaps the experimental results can be summed up most adequately by means of a velocity of reaction-temperature graph.

In Table VI are given the values for the amount of reaction per minute before the reaction has proceeded to half-value, at the various temperatures at which experiments have been carried out.

**The experiments were all carried out at pressures in the neighborhood of 100 atm. It may be possible that a homogeneous liquid phase could be obtained at much higher pressures, due to increased solubility of hydrogen chloride in the liquid phase.*

TABLE VI

VELOCITY OF THE REACTION BETWEEN PROPYLENE AND HYDROGEN CHLORIDE AT DIFFERENT TEMPERATURES

Temperature, °C.	−78.5	0	+20	+25	+45	+50	+75	+78	+80	85	+105
Velocity of reaction, % per min.	0	0.15	0.21	0.23	0.31	0.33	0	0	0	0	0

In the graph, Fig. 6, a curve is drawn through the experimental points and the dotted portions include an area within which the curve must lie. In the region between 50 and 74° C., measurements that would have the same quantitative significance could not be made. The two dotted portions indicate however the extremes within which the curve representing the reaction in a homogeneous phase must lie.

Discussion

It was pointed out in the introduction that a corollary to the hypothesis that a chemical reaction is dependent on either a molecular attraction between the reactants or where this does not exist, dependent on a catalyst, is that with rise in temperature the velocity coefficient of such a reaction might diminish and even become negative. A more detailed discussion of this is given by Maass and Sivertz (9, p. 2889). The question arises whether the results obtained in this research give evidence of such an inversion in the temperature coefficient It must be kept in mind that the hypothesis does not demand an inversion, but simply requires an influence contrary to the effect of activation, which latter may be so large as to mask the other influence. Therefore the absence of an inversion does not necessarily disprove the case.

Coming now to the experimental results; if the velocity coefficient of the reaction in the liquid state had shown a decided decrease at the higher temperatures, not much further need be said. All that has been shown definitely however is that in the liquid state the velocity of the reaction increases in the region where quantitative results are obtainable and becomes zero above the critical temperature, although no discontinuity in concentration takes place under the experimental conditions. It might therefore be concluded that the velocity coefficient of the reaction acts normally in the liquid state with change in temperature, and that the change in state precludes a reaction occurring. The hypothesis therefore which is justified is a modified form, and may be stated briefly as follows. *In the liquid, regional orientation takes place, especially where in a binary mixture the two species of molecules have a strong attraction for one another. This regional orientation is of great advantage in furthering the reaction. With rise in temperature the thermal agitation decreases the extent to which this regional orientation takes place. The increased activation of the reacting molecules with rise in temperature more than compensates for this, until the critical temperature is reached. At the critical temperature there is a very rapid decrease in regional orientation resulting in an equally rapid change in the velocity coefficient.*

It is realized that the above views may appear rather radical, as involving almost a discontinuity in state at the critical temperature. This might be considered contrary to the parallelism of the pressure-volume isothermals, one just above and one just below the critical temperature. The drastic change in regional orientation which is assumed to take place in the neighborhood of the critical temperature, while obviously of great influence in governing the reaction velocity, need not necessarily however greatly influence the pressure-volume relationships of the two isothermals mentioned above, and thus not give rise to an apparent discontinuity in state.

As an analogy to the above statement the phenomenon of the so-called "liquid crystals" may be mentioned. The turbidity and optical properties of these substances undergo a rapid change in a very small temperature region without any appreciable change in the other physical properties. This is due to a pronounced regional orientation (1, 2, 3) where exceptionally polar molecules are in question. The regional orientation in the liquid is a resultant orientation of the liquid molecules differing from the perfect and fixed orientation of the atoms in a crystal, inasmuch as the molecules retain their identity and rotational and kinetic energy in the case of the liquid. The average molecule in a certain group only is orientated with respect to its neighbors. The sharp change in turbidity in the case of the liquid crystals without corresponding sharp change in physical properties has been satisfactorily explained by Nernst (13) on the basis of a sudden change in regional orientation.

That regional orientation exists in all liquids to a greater or lesser extent is a possibility. That it exists in highly compressed gases is a further possibility. That a marked change in the extent of regional orientation is closely associated with the critical phenomena is the speculative viewpoint of the authors, inasmuch as it is useful in explaining the results of the experiments.

The ideas which have been developed are necessarily subject to modification in the light of further investigations. These ideas however, if nothing else, are useful in suggesting a considerable amount of research which is to be undertaken in this laboratory. Already a research is under way in which the critical phenomena are being investigated, and some interesting results have already been obtained with regard to the lag in equilibria in a one-component system at the critical temperature. The particular reaction between propylene and hydrogen chloride is to be examined in an inert liquid medium at temperatures above those reached in the present investigation, and also other systems are to be examined. The effect of the presence of other components on the course of the reaction is to be investigated. It may well be for instance that, where a number of simultaneous reactions occur, the fact that above a certain temperature some of them may cease, will be of considerable practical value.

References

1. Bose, E. Physik. Z. 9: 708-713. 1908.
2. Bose, E. Physik, Z. 10: 32-36. 1909.
3. Bose, E. Physik. Z. 10: 230-244. 1909.
4. Coffin, C. C. and Maass, O. Can. J. Research, 3: 526-539. 1930.

5. Coffin, C. C. and Maass, O. Ph. D. thesis, McGill University.
6. Coffin, C. C., Sutherland, H. S. and Maass, O. Can. J. Research, 2: 267-278. 1930.
7. Maass, O. and Russell, J. J. Am. Chem. Soc. 40: 1561-1573. 1918.
8. Maass, O. and Russell, J. J. Am. Chem. Soc. 43: 1227-1230. 1921.
9. Maass, O. and Sivertz, C. J. Am. Chem. Soc. 47: 2883-2891. 1925.
10. Maass, O. and Sivertz, C. Ph. D. thesis, McGill University.
11. Maass, O. and Wright, C. H. J. Am. Chem. Soc. 43: 1098-1111. 1921.
12. Maass, O. and Wright, C. H. J. Am. Chem. Soc. 46: 2664-2673. 1924.
13. Nernst, W. Z. Elektrochem. 16: 702-707. 1910.
14. Sutherland, B. P. and Maass, O. Trans. Roy. Soc. Can. 20, III: 499-505. 1926.

THE USE OF ARTIFICIAL ILLUMINATION FOR GRADING GRAIN[1]

BY D. C. ROSE[2]

Abstract

The first part of this paper is a description of artificial lighting units designed to give a suitable illumination for grading grain. Two types of illumination are being tried; first, the imitation of daylight by means of daylight lamps; second, the use of colored lights which emphasize the bad and good points in wheat. A combination of a mercury lamp, neon lamp and the General Electric type S1 sun lamp gives promise of being a satisfactory source of illumination of the second type. A grain grading unit of each type is being given a prolonged trial.

The second part describes experiments which were an attempt to find a more objective means of grading wheat. The light reflected from wheat of different kinds and different grades was analyzed both spectroscopically and by means of a photo-electric cell and light filters. In the spectroscopic measurements ultra-violet light was included. The results indicate a certain amount of selective reflection but the variations with the different grades are not of a nature which would be helpful in grading wheat.

There are two important reasons for the adoption of artificial illumination for grading grain. First, some difficulty has been experienced with the present practice in that a sample of wheat is sometimes given a different grade when examined at inspection points some distance apart. It is quite probable that many such disagreements in the grades are due to the differences in illumination. There is also a personal element which probably can never be entirely eliminated unless some physical means of grading are found. The use of a uniform type of illumination should reduce such errors to a minimum. Second, during rush seasons there is often delay at elevators and in transportation as the grain cannot be binned or shipped until it has been graded. Often in dull weather there are only a few hours of daylight suitable for examining the grain and costly delays occur. If artificial lighting were used these delays would be eliminated and in rush seasons grading could be carried on all night.

In this report are described lighting units which the author erected in the Laboratories of the National Research Council in Ottawa, with the object of having them tried out by grain inspectors. Two very different types of illumination show prospects of being satisfactory. A description is also given of an attempt to use other physical means of grading wheat.

Part I

Sources of Artificial Illumination

Requirements

The requirements for the illumination of a grain inspection table are slightly more exacting than, though not dissimilar from, artificial illumination problems where color matching is required. The requirements may be put under three

[1] *Manuscript received June 19, 1931.*
Contribution from the National Research Laboratories, Ottawa.

[2] *Assistant Research Physicist, National Research Laboratories, Ottawa.*

heads: (a) color or composition of the light, (b) intensity of illumination, (c) diffuseness of the illumination.

Of these three qualities it is difficult to say which is the most important. One usually assumes that for color comparisons the first is by far the most important, but there is considerable evidence that the other two are equally important and in fact there is some evidence that intensity is rather more important than exact color or composition of the light, provided it does not differ enormously from white light. This point will be brought out below.

The choice of a source of illumination was considered from two points of view. First, the imitation of daylight was obviously a step in the right direction since it is the type of illumination ordinarily used for grain inspection. Second, it was decided to try to produce an inspection cabinet or booth in which the illumination would come from lights of different colors, which would make the more important blemishes as well as the good points in wheat appear more prominently than with daylight.

The details as to the best intensity, diffuseness and color of light to be used for grading grain were obtained only after a number of lighting arrangements had been tried, so it seems advisable to describe at this point some of the lighting units tried and then discuss the requirements under the three heads given above. In order to make as little change as possible in the routine of grain inspecting, it was necessary to make the inspection table or space as nearly similar as possible to that already in use. The usual inspection table is a bench about two to three feet wide placed directly in front of a large north window. As far as the author is aware, there is no definite rule regarding the size or height of the window, but it must not be shielded by other buildings and must be sufficiently large to illuminate uniformly an area on the bench, two to three feet square, and with sufficient intensity for the inspector to be sure of his grade.

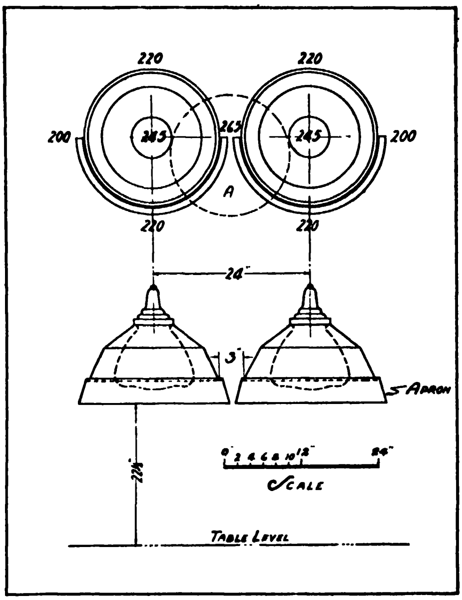


FIG. 1. *Daylight units consisting of two Ivanhoe Glassteel Diffusers with "Trutint" globes. The figures in the plan represent the approximate intensity on the table in foot-candles. The circle A is the best place to examine the wheat. The inspector should hold his head above the place marked A.*

Daylight Units

The most usual type of daylight unit is either an ordinary gas-filled incandescent lamp, the bulb being made of a special blue glass, or an ordinary clear glass bulb covered by a globe made of special blue glass. The object of

the blue glass is to absorb the excessive red and yellow rays produced by the incandescent filament. This, of course, reduces the efficiency of the lamp somewhat. There is an enormous number of such fixtures of various shapes and sizes on the market (1, p. 302), a large number of which would obviously be unsatisfactory. Before settling on definite units to be tried, a consultation was arranged with Mr. J. W. Bateman, Manager of the Lighting Service Department of the Canadian General Electric Co., Toronto. The experience gained by this Department was found to be invaluable and was largely responsible for the selection and arrangement of the lighting unit which will probably be most satisfactory for grain inspection.

For direct lighting units several types of metallic reflectors were tried, but the two most satisfactory were found to be, first, a pair of Ivanhoe Glassteel Diffuser fixtures (500 watts each) with a "Trutint" glass globe. Two such fixtures form one lighting unit arranged as in Fig. 1. Second, a pair of aluminium high mounting units (500 watts each) using Mazda daylight lamps gave a good diffuse illumination, but the color was not as good as with the Trutint globe, so it was not considered necessary to give them a prolonged trial. They are merely mentioned as being a second best. The Glassteel Diffusers were, however, set up in Winnipeg for a prolonged trial.

In order to obtain the required intensity, it was necessary to hang the Glassteel Diffuser fixtures about 22 in. from the table. With the fixtures at this height, a grain inspector, if he looked up, could see the bottom of the globe. This arrangement would cause a certain amount of glare in his eyes which it was desirable to eliminate; an additional apron was therefore added to the shade. This apron extended only half way around the fixture so that the inspector whose eyes are normally about on a level with the bottom of the apron would receive no glare from the opposite side of the shade.

Colored Light Sources

Examination of a sample of wheat under an intense mercury vapor lamp gave a surprising result. The green and frozen wheat showed up much more plainly than in daylight. Starchy kernels could also be distinguished though not quite as easily. The good grain was least prominent, which is not surprising as the mercury spectrum consists mostly of strong yellow, green, and blue violet lines. The frozen wheat seems to reflect a relatively higher portion of blue and violet light than good or starchy kernels. Hence this property is magnified by the excessive intensity of light of that color. Green wheat is made prominent by the intensity of the mercury green line, but this light alone would not do for grading as it suppresses the good points of the grain. The wheat was also examined by light from an intense neon lamp, which, being nothing but an intense red light, made all the wheat grains appear red, signifying a better grade than it appeared to be in daylight. This light alone would be useless for inspection purposes because the green wheat would not show up at all and frozen wheat could be distinguished only by its scaly surface.

Another source of illumination which has been receiving attention recently

is the General Electric Type S1 Sun Lamp. This consists of a mercury arc between tungsten electrodes. The electrodes become incandescent and the general illumination is not unlike a Mazda lamp, but includes in addition the mercury lines in the ultra-violet. The lamp is made in a special ultra-violet transmitting glass. The addition of some ultra-violet light to the illumination of an inspection table for wheat seemed a possible improvement due to the fluorescence it produces, though this detail was not investigated separately.

A large number of combinations of the following three lamps and daylight lamps were tried:

1. Cooper Hewitt Mercury Vapor Lamp M Tube Type, 110 volts a.c., 450 watts.
2. Cooper Hewitt Hot Cathode Neon Lamp, 110 volt a.c.
3. General Electric Type S1 Sun Lamp.

Finally an experimental grain inspection cabinet as shown in Fig. 2 was built.

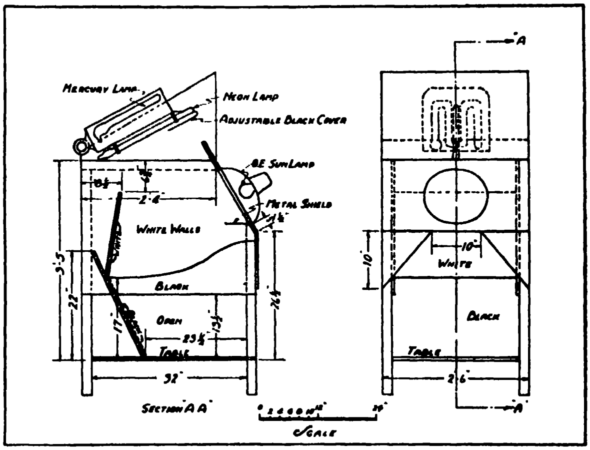


Fig. 2. *Inspection cabinet, containing mercury vapor lamp, neon lamp, and the General Electric Sun Lamp. Most of the supporting framework is omitted and only the important dimensions are given.*

The neon lamp was attached to the reflector containing the mercury vapor lamp. These were placed at the top of the cabinet, the light coming from them directly to the inspection table and being reflected from the white parts of the side walls. The General Electric Sun Lamp was shielded from the table and so placed that the lighting from it was indirect. The relative intensities of the three could be varied considerably by raising, lowering or tipping the reflector containing the mercury and neon lamps, covering the neon lamp, or varying the amount of white and black in the walls of the cabinet. The arrangement shown in Fig. 2 seemed most satisfactory and this unit was set up in Winnipeg for a prolonged trial.

Color and Intensity

As these two properties of illumination are to some extent complementary, especially with regard to this problem, it is difficult to discuss them separately.

First, with regard to color, it has been stated above that the lighting used normally for grain inspection is north skylight. The color temperatures of various types of daylight, as given by Luckiesh (2), are shown in Table I.

The light on the inspection tables in the inspection room of the Board of Grain Commissioners in Winnipeg was examined by the author for two days, and though no colorimetric measurements were made, some idea of the variation

TABLE I

COLOR TEMPERATURES OF VARIOUS TYPES OF DAYLIGHT

Type of daylight	Approximate color temperature, °*K*
Direct sunlight before 9 a.m. and after 3 p.m.	4400 to 5000
Direct sunlight 9 a.m. to 3 p.m.	5300
Sunlight plus light from hazy or smoky sky	5700
Sunlight plus light from clear sky	5800
Light from totally overcast sky	6400
Light from hazy or smoky sky	7000 to 8000
Light from clear blue sky	10000 to 20000

can be obtained from the above table. As the room is on the eleventh storey, and there are no neighboring buildings nearly as high, the light, with the exception of that from a ledge in front of the windows, comes from the north sky. On clear days with blue skies this has a color temperature of from 10,000° *K* to 20,000° *K*. On overcast days it might be about 6400° *K* as Table I indicates. The relative luminosity of violet and blue light at these color temperatures according to Luckiesh is 166 to 115 respectively; for green light, 116 to 105; for yellow light, 92 to 98; and for orange and red light, 75 to 93. The reversal in order of these figures gives an idea of the change in color even under best inspection conditions.

Further, it is quite possible that even a greater variation in color may be found. The intensity of illumination was measured at various times throughout the day for two days. The measurements were not very accurate as they were taken with a Macbeth Illuminometer with no color filter. However, the error in relative variation is certainly not greater than 6 or 8%. The results are plotted in Fig. 3. The increased intensity around 10 to 12 a.m. was probably due to a horizontal ledge, about 10 ft. wide, nearly level with the window sills and forming a part of the roof of the storey below. This ledge reflected a considerable quantity of direct sunlight which no doubt accounts for some of the increase in intensity at the top of the peak. Towards afternoon this ledge was in the shadow of the roof and the intensity dropped. The effect of this ledge would also decrease the color temperature of the light somewhat, thereby making it more yellow. Of course there is a natural variation in the intensity of daylight as well as in the effect of the sun on surrounding objects.

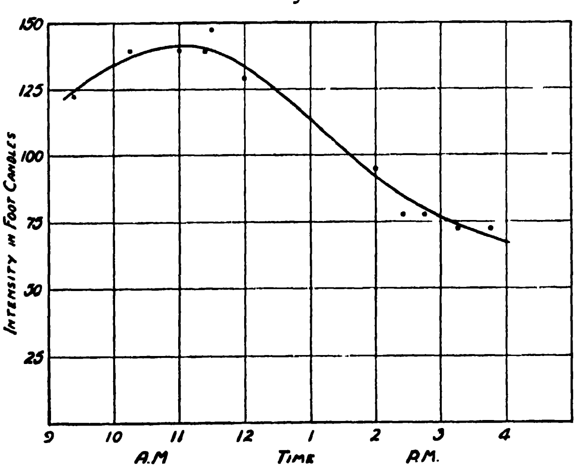


FIG. 3. *Intensity of illumination measurements on one of the grain grading tables in Winnipeg. Observations were taken on two clear days.*

Thomson (3) has made some measurements at the Manitoba Agricultural College in Winnipeg of the variations in intensity of daylight, that is, the total radiation coming from the sky in all directions. His results show curves not unlike that in Fig. 3. The intensity of north skylight shielded from the sun would not be expected to vary as much throughout the hours in Fig. 3 as total daylight, but the variations would be similar. He found that sometimes when the sky was patchy the intensity would increase for a short period as much as 50% above that for a clear day. Also the difference in intensity between a clear day and a dull day was very great. His curves were taken in winter and as they represent total daylight, cannot fairly be taken as representative of conditions on the grain inspection table. However, it may be concluded without doubt, that intensity variations occur in the illumination on the inspection tables which would probably be between a small fraction of the intensities shown in Fig. 3 to double the highest point on the curve. Such variations would exist under different weather conditions even between the hours of 8 a.m. and 4 p.m. during which most of the inspection work is carried out.

Thus we see enormous variations in both color and intensity of natural light, all of which would be avoided if artificial lighting were used entirely for grain grading.

It is well known that the color discrimination of the human eye depends greatly on the intensity of illumination on the object being observed. There is probably an optimum intensity at which the color discrimination as well as the form discrimination is a maximum. Form discrimination, that is, ability to see quickly the shape of the kernels as well as roughness of the surface, etc., is important in picking out green and frozen wheat. The intensity of illumination at which color and form discrimination are best for wheat inspection was found to be about 200 foot-candles. Anywhere from a little under 200 to 250 foot-candles seems equally good, though the best intensity probably varies somewhat with the individual. This intensity is considerably higher than is ordinarily used even for close work, but is compensated for by the low reflecting power of the wheat. Above 250 to 275 foot-candles the illumination is somewhat too bright for comfort. These observations of the best light intensity have been confirmed by engineers in the Lighting Service Department of the Canadian General Electric. It cannot be determined quantitatively with any great accuracy but the above figures were obtained by finding the intensity at which color and form discrimination was easiest for the observer. The best intensity would be expected to vary considerably with the object being examined and with the background, and probably is near the highest intensity at which there is no eyestrain.

The color of the lamps now being tried is not exactly that of north skylight. The light from the daylight units closely resembles the color of noon sunlight. The color of the light in the cabinets (Fig. 2) should not be compared with daylight as the illumination given by these lamps is mostly concentrated in certain wave-lengths. The Sun Lamp gives considerable illumination of the

continuous spectrum type but the light from the mercury and neon lamps is concentrated in the characteristic spectra emitted by these elements.

One of the problems in color-matching of any sort is to define exactly what light is to be used for a standard. Obviously, merely the term "daylight" will not do as it varies so much, and the term "white light" is also arbitrary and in itself is indefinite. The General Electric Company and others interested have done a great deal of work on this subject. They seem to have taken noon sunlight for the standard white light. Average noon sunlight has a color temperature from 5000° K to 5400° K and this color represents a good standard for white light. The color temperature of the Ivanhoe Trutint units similar to those being tried in Winnipeg is given by Luckiesh as 6200° K, so they are slightly more bluish than average noon sunlight. It is expected that this unit will prove satisfactory for grain inspection.

From data given above and the results of preliminary tests described below, it is evident that grain can be, and is being, inspected in a satisfactory manner in light of any color temperature from about 4500° K to 15000° K. It is also probable that the inspection cabinet described above, which gives mainly a line spectra source of light to which no definite color temperature can be applied, will prove satisfactory for grain inspection.

From these observations and those of others, it is becoming increasingly evident that in such work as the grading of wheat, the color of the light is if anything less important than its intensity and diffuseness, provided, of course, the color does not differ enormously from, say, noon sunlight. The author is convinced that light from an ordinary Mazda gas-filled lamp properly diffused and of the right intensity could be used for grain inspection, though it would be necessary for the inspector to undergo a period of training in order to grade correctly with such lighting. However, it is expected that, with the lighting units now installed in Winnipeg, no such period of training will be necessary, though it is not considered advisable to have an inspector constantly changing from artificial to natural light.

Diffusion

The diffuseness of the illumination has been mentioned several times without any definite measure of this quality being given. Actually no quantitative measure of diffuseness was made. It is advisable, where an assembly of small objects like grains of wheat are to be examined, to have the light coming in from all sides as well as from above. The main object of using two Glassteel diffusers instead of one was to give the correct diffuseness as well as a sufficiently large area of illumination (Fig. 1). The light coming to the inspection point marked comes mainly from above and from both sides. In the cabinet it was easy to have the light quite diffuse as the sources of illumination were large. The indirect components from the sides of the cabinet make the illumination so diffuse that no definite shadow can be seen when the hand is held as close as four or five inches from the table. The daylight units were almost as good.

Good diffusion is necessary for the examination of grain because the shape of

the kernels is very important, particularly in the detection of different kinds of wheat, foreign matter and immature wheat. A good diffuse light leaves no sharp shadow and no intense highlights where specular reflection takes place. The amount of specular reflection in wheat, although it has not been measured, is small. The condition of the surface of the kernel gives the indication as to whether or not it has been frost bitten.

Grading Tests with Artificial Light

While the author was in Winnipeg some tests were made using the two lighting units described in Fig. 1 and 2. The procedure was as follows:—

The grain was inspected and given a grade in the normal manner using natural daylight. This was considered to be the correct grade. Then the sample was passed on to the inspector using the inspection cabinet, without informing him of the grade already given. Here it was again graded and then passed on to a third inspector working under the daylight lamps. Forty samples of wheat were used, and a comparison of the sheets on which the grades were tabulated showed that the grades given under the daylight lamps were all correct. Thirty-four out of the forty were correct when graded in the cabinet. Two of the other six were doubtful, as they were on the border between 1 Northern and 2 Northern, but the inspector before finishing with them gave them the correct grade, leaving four still wrong. These were graded one grade too high. For this test the mercury and neon lamps were raised a little and tilted higher than in the position shown in Fig. 2, causing the light on the table to become redder and of about 150 foot-candles intensity. The lamps were returned to their normal position and the four samples re-examined and a correct grade was given. This, of course, can be considered as only a preliminary test. The units are being given a prolonged trial in Winnipeg and more complete reports will no doubt be available later.

Inspectors' Hours

The use of artificial light for inspection purposes is bound to cause a slightly greater nervous strain on the inspector, due more to the fact that he may be in a closed room or working at night than to any eyestrain caused by the lights. With this in view, a series of directions to inspectors have been drawn up by the author. It should be emphasized that these are only tentative and if grading is carried on at night, the periods of rest and hours of work adopted would have to be decided by those more familiar than the author with actual conditions in the elevators. These are merely offered as a working plan. The first two articles refer particularly to the cabinet containing colored lights. These should be adhered to strictly for the cabinet unit, if good results are to be obtained. However, an inspector should be able to change from daylight to the daylight lamps without much difficulty.

Directions to Grain Inspectors Using Artificial Light

1. Inspectors who grade grain by artificial light should use artificial light only and should not grade by any other light either during the day or night.

2. Should it be necessary to change an inspector from artificial light to daylight inspection or *vice versa*, the inspector should practice for a period to be

determined by the Board of Grain Commissioners or the chief inspector, with whichever light he is adopting before his word is taken as final.

3. An inspector should not grade continuously for a period greater than three hours without at least an hour's rest, and should not grade for more than two or at the most three such periods per day.

4. Recreation and fresh air are suggested between periods.

5. Under rush conditions these periods might be run up to four three-hour periods for a limited time, provided the inspector is in good health.

6. If it was found suitable, four two-hour periods with half-hour and hour intermissions taken alternately might be adopted. The best periods and hours can be found only by experiment and will probably vary for different men. Application of such a regulation would without any doubt improve the accuracy of daylight grading.

7. Inspectors should avoid looking directly at any of the lamps used for illumination, it being impossible to shield them completely.

8. If there is any tendency to eyestrain, the inspector should look around the room at distant objects for a few minutes. Five minutes' walk outside would be very good but if taken at night or on dark days, inspection should not be commenced for ten minutes after the intermission.

General Illumination of Grading Room

The general illumination of the inspection room is exceedingly important if artificial lighting is to be used successfully for grain grading. The requirements however are not very severe. The general illumination anywhere in the room should be between 20 and 40 foot-candles at the level of the table. This is rather a high level for artificial illumination but in order to reduce the intensity contrast between the inspection table and the rest of the room it should not be much less than 20 foot-candles. It must also be diffuse, for it is necessary to prevent bright highlights from any objects in the room. Indirect lighting with large illuminating surfaces would be best but is probably too inefficient for practical purposes. Glassteel diffuser units are probably next best, as the large globe and reflector diffuse the light sufficiently so that they can be looked at directly without straining the eyes. The whole room should be illuminated as uniformly as possible and the ceiling and walls down to about eye level should be white, and below that, some dull neutral color. The ventilation must be good and the room kept clean and attractive. This is sometimes very difficult in elevators, but if reliable grading is to be obtained considerable attention must be paid to comfortable working conditions for the inspector. This applies particularly to artificial lighting when used at night.

When grading is being done at night some eyestrain will be experienced when an inspector comes into the inspection room from outside. Owing to the high level of intensity necessary in the room, a man coming in from outside, having his eyes adapted to the dark, would find the light exceedingly brilliant and would be able to see nothing for several minutes. The strain involved in coming from darkness to a brilliantly lighted room is sometimes painful unless the individual is trained to shield his eyes and spend five or ten minutes in

getting used to the bright lights gradually. Inspection of wheat should not be attempted until the inspector has been in the lighted room for 15 to 20 min.

Location of Artificial Lighting System

The location of the lighting units, particularly the daylight units, is a question of some importance. It is not advisable to use them on the same table that is used for daylight inspection. If the hanging lights (daylight units) are near the wall, it will have considerable influence on the illumination of the table, as it acts as a source of indirect lighting. If hung in front of a window (*i.e.*, using the same table that is used for daylight inspection) a black blind drawn over the window at night would not interfere with the lighting but it would limit the inspectors' range of vision considerably. If when the inspector looks up from his work he can see only a wall immediately in front of him, fatigue will be noticed quicker than if the wall was ten or twelve feet away. It is for these reasons that it is recommended that the table for artificial light inspection be in the centre of the room rather than at the wall.

The position of the cabinet if it is adopted is not so important but should be arranged so that when the inspector turns from the cabinet to write down the grade his limit of vision should not be cramped by a nearby wall.

Part II

Grading by Physical Means

The object of these experiments was more to assist grain inspectors in grading wheat than to produce a purely physical method of determining the quality of wheat. Obviously the best method of giving a grade to a sample of wheat would be based on a milling and baking test, but such a test would require too much time. Actually the inspectors are trained to detect very quickly the imperfection which would spoil milling or baking and the process of actually giving a grade after the separation of foreign matter, etc., takes only about half a minute. It was hoped that an examination of the light reflected from the surface of a pile of wheat might yield a simple objective method of detecting the quantity of such blemishes as frozen grain, starch, etc. The results will be seen to indicate that there is insufficient selectivity in the reflection or scattering of light to be of any valuable help in estimating the amount of, say, frost bitten wheat present. The experiments indicate that the human eye is a great deal more sensitive in color and form discrimination than a photo-electric cell.

The problem was attacked by two methods. First, the intensity of the light reflected from various samples of wheat was measured by a photo-electric cell using Wratten filters to separate the colors. Second, the light reflected from the samples was examined spectroscopically and the spectrograms were measured photometrically to give the relative intensities at various wave-lengths.

Measurements with Photo-electric Cells

The arrangement of the source of illumination and the box containing the

wheat and the photo-electric cell was usually as shown in Fig. 4. The source of illumination used varied with the color of light being studied. For the red part of the spectrum the General Electric Sun Lamp was used as it was found to operate very steadily. Owing to its construction and way it is connected with its regulating transformer, small variations in line voltage have but little effect on its intensity. For the blue and green light a mercury vapor lamp was used.

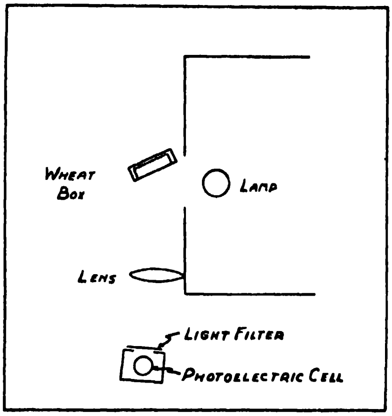


FIG. 4. *Arrangement of apparatus for measuring the intensity of reflected light from various kinds of wheat.*

The box containing the wheat was about 3 by 1½ by ½ in. and had a plain glass front which was kept clean. The box was so orientated that no specularly reflected light from the glass could reach the photo-electric cell. The light filters were placed directly in front of the photo-electric cell as shown.

The color sensitivity of the combination (photo-electric cell, filter and source of illumination) was checked by means of the Hilgar wave-length spectrometer, so as to make sure the light being measured was of the right color. The General Electric Sun Lamp, or for that matter any incandescent lamp, could not be used as a source of green and blue light as all the Wratten filters used transmit infra-red light and the photo-electric cell was sensitive some distance in the infra-red. At the time the experiment was performed no cells which were not sensitive in the red were available. Fig. 5 shows the results.

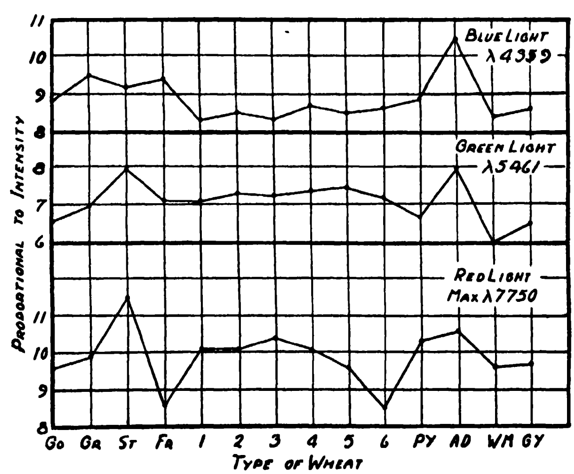


FIG. 5. *Variation in intensity of light of different colors reflected from various samples of wheat. Intensities measured by means of a photo-electric cell.*

The types of wheat sampled were: (a) Four samples picked by hand. They consisted of good wheat (Go.), practically perfect kernels; green wheat (Gr.), immature but not frost bitten; starchy wheat (St.), well filled starchy kernels; and frost bitten (Fr.), fairly well filled kernels with a heavy bran frost. (b) Then the six grades of wheat, numbered in the curves from 1 to 6, were tried. These samples were sent to the author by the inspection department of the Board of Grain Commissioners in Winnipeg. (c) Good samples of four other types of wheat were also used;

Parkers Yellow (PY); Amber Durum (AD); White Marquis (WM); and Garnet Yellow (GY).

An examination of the curves in Fig. 5 shows a variation of the type one would expect. First using red light, the good and green wheat reflect nearly the same quantity; the starchy, over 10% more and the frost bitten, less. With green light the relative intensity from starchy wheat was reduced and that from frost bitten wheat increased. With blue light (wave-length 4359Å) the intensity of light from starchy wheat was less than frost bitten. Owing to the yellow color of starchy wheat one would expect greater intensity of the reflected light in the red and green. An examination of the curves representing light from the six grades is also as would be expected. No. 1 Northern and No. 2 Northern were about the same, No. 3 Northern contained more starch and No. 4, 5 and 6 contained more frost bitten wheat as the grade became poorer. No. 6 was largely frost bitten wheat. The variation in intensity of green and blue light from the different samples is less than of red.

The other types of wheat show no variation of interest except that Amber Durum reflects relatively more light of all colors, particularly blue. It should be pointed out that the vertical axis of these curves represents the current in the photo-electric cell. The different curves cannot be compared as the different filters absorb different fractions of the incident light.

The accuracy of these curves is not very great. To check it a sample of wheat was put in the box and a reading taken, the wheat then taken out and poured in again and another reading taken; this was repeated many times and considerable variation in intensity was found. A set of readings obtained was as follows; 8.9, 9.6, 9.4, 8.5, 9.5. This shows a variation of over 10%, nearly as much as the variations in the curves in Fig. 5. However, as Fig. 5 represents the average of a large number of runs they are more accurate than that. This variation was not due to variations in the intensity of the illumination nor in the position of the box because these points were checked regularly.

Spectroscopic Measurements of Intensity of Reflected Light

Fig. 6 shows a plan of the apparatus used for this experiment. The object of using spectroscopic methods was to carry the investigation into the ultra-violet. However, some spectra were taken in the visible using a Hilgar wave-length spectroscope. The blackening of the plates was measured by a Zeiss microphotometer and the intensity of blackening plotted.

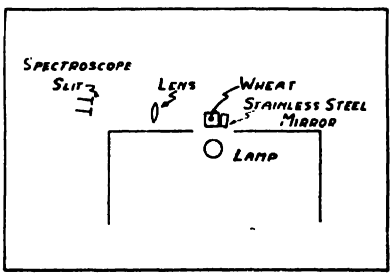


FIG. 6. *Arrangement of apparatus for photographing spectrum of reflected light from wheat.*

In Fig. 7, "A" represents the results in the visible part of the spectrum. Using visible light, the light from a small pile of wheat was reflected by a mirror and lens into the slit of the spectrograph. As high lights and low lights always exist in such a pile of wheat the lens was arranged so that the image of the

wheat did not focus on the slit. Different sources of illumination were used to bring out different parts of the spectrum. The results in Fig. 7 "A" were obtained with an iron arc and the General Electric Sun Lamp. The wave-lengths bracketed were on the same plate. The ordinates are not given numerically as the plates were not calibrated. They represent the deflection of the electrometer fibre in the microphotometer. This bears a relation to the intensity of the light on the plates somewhat similar to a logarithmic curve, so the sensitivity of the method was considerably greater for faint spectral lines than for strong. The results agree with those taken with a photo-electric cell. The starchy wheat reflects relatively more red and yellow light than blue, while there is not so much difference between the other samples.

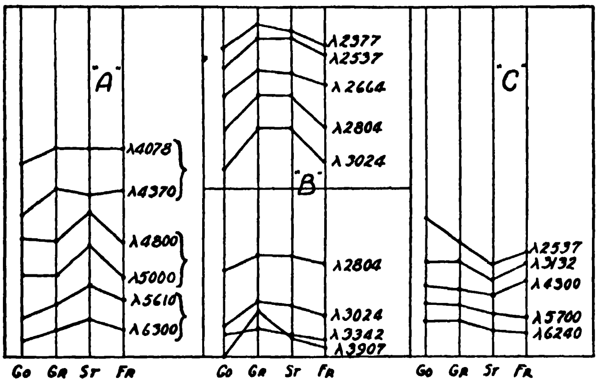


FIG. 7. *Variation in intensity of light of different colors, including the ultra-violet, reflected from samples of good, green, starchy and frost-bitten wheat. Intensities measured by spectral photometry. The wave-lengths are given in Ångstrom units.*

Some variations show up with good and green wheat but not too much attention should be paid to these for reasons explained below, which make these spectroscopic results decidedly inferior to those taken with a photo-electric cell.

In Fig. 7, "B" and "C" represent the results taken with a quartz spectrograph. A quartz mercury vapor lamp was used for "B", and "C" was obtained partly with the same lamp and partly with a cored carbon arc. The results appear somewhat erratic. In this case a single grain of wheat of the type investigated was used as the sample. This was placed on a black cloth under the lamp and the light from it focused on the slit of the spectrograph by means of a stainless steel mirror and a quartz lens. The method used with visible light, that is, using a pile of wheat out of focus, did not work at all satisfactorily with the ultra-violet spectrograph and in fact the single kernel method was not much better. An examination of the spectrograph plate showed, first, that there was no definite band of selective reflection which would be useful in grading and, second, that even taking great precaution in getting uniform illumination of the wheat, owing to the rough uneven nature of the surface of the kernel, it was practically impossible to get a uniform portion of its image on the slit without using magnification which would make the exposures unreasonably long. As it was it took half an hour to obtain an exposure. The light was kept constant by directing a portion of it into a photo-electric cell and adjusting the current in the arc to keep the cell current constant.

Again in Fig. 7, "B" and "C", the zero of the ordinates is not shown and so only the general shape of the curves can be compared directly. For instance

the lines λ3907 and λ3342 show marked differences but actually the intensity variation was in about the same proportion, λ3907 being a much weaker line than λ3342. The difference in intensity of the light reflected from good and green wheat here would be about 15 or 20%.

As mentioned above, the slit was not uniformly illuminated, so the measured intensity of the lines would be different for different parts of the slit. An example of this is shown in the case of the lines λ3024 and λ2804 which were run through the microphotometer twice. The shape of the curve is in general the same but the variation in intensity is different. In Fig. 7, "C" further amplifies the result of irregular illumination of the different types of wheat. Here even with red light the intensity variation does not agree with that found when photo-electric cells were used. This is no doubt due to the fact that illumination of the starchy kernel was bad. To overcome this trouble and obtain consistent and accurate results would have involved the use of much more elaborate apparatus and as there is definitely no hope of using photometric methods of this sort for assistance in grading, further experimental work was not considered worth while.

The curves in "B" and "C" do, however, show some consistency with those in "A" and the measurements with a photo-electric cell. If the intensities of reflected light from the various types of wheat had been plotted in proportion to that reflected by, say, good wheat, the curves in Fig. 5 and 7 would be the same shape. Even in Fig. 7 "C" the relative intensity of light reflected from starchy and good wheat is greater for red light (λ6240) than for violet (λ4300). The line λ2537 appears in both "B" and "C" and the variation is quite different. This is also due to the difficulty of illumination.

Conclusion

Regarding the first part of this paper on the use of artificial light for grading, there is little to say except that, without doubt, artificial lighting can be used in a satisfactory manner for grading grain and from a purely physical point of view it should be better than natural light. One of the main objections to it, and probably the only real one, is psychological. Many individuals who are used to outdoor life and working under natural light find some discomfort in working in closed rooms using artificial light. While a grain inspector is not an outdoor man his training with natural light is so long and rigorous that a sudden change may cause real or imagined discomfort. This should be overcome to a large extent by keeping the room in which the inspector is working as pleasant as possible, well and uniformly illuminated and well ventilated and of course at comfortable temperatures.

Quite likely a considerable preference for artificial light would develop in inspectors who used it for some time. Many individuals who do a great deal of close work much prefer artificial light to daylight owing to its greater steadiness and the ease in locating the light where it is wanted.

The relative importance of intensity, color and diffuseness are fully discussed above so little more can be said here.

Regarding the second part, grading by physical means, the method tried did not give results which would be of any use. Consider the curve in Fig. 5 for red light. Supposing an unknown sample of wheat were put in the box and the current in the photo-electric cell gave a 10.1 for a reading. From the curve one would not be able to tell whether the sample were No. 1 Northern, No. 2 Northern, No. 4 or a mixture of Amber Durum and White Marquis, or probably a dozen other kinds of wheat.

If the curve for grades from one to six had no reversal in slope then the results might have been useful and some sort of a colorimeter could be devised to help grading. The conclusions that can be drawn are that the grade is given more by the shape of the kernels and the nature of the surface than by the actual color. Of course color is extremely important but in the grading of grain it cannot be separated from form discrimination. In good, starchy and green wheat the color is more that of the inside of the kernel, while in frost bitten wheat, the color is more that on the surface.

Numerous blemishes other than those examined and numerous other types of wheat were omitted from the observations in order to correlate the results with known variables.

Other physical methods might be developed but it is doubtful if they would be any better. Grinding the grain before performing the experiments described in Part II might have produced more consistent results but they would not likely have been any more useful. If the exact properties of wheat which make it good for grinding and milling were better known, something more might be done to use physical means of grading. Chemical tests for protein, etc., take too long to be of any use and in any case the protein content of wheat does not seem to follow the grade in any regular manner. The only improvement that is obvious at present is the use of artificial light for examination.

References

1. Cady, F. E. and Dates, H. B. Illuminating engineering, Wiley, 1925.
2. Luckiesh, M. Elec. World, 95: 263-264. 1930.
3. Thomson, W. A. Can. J. Research, 4: 559-564. 1931.

THE PROBLEM OF THE ELECTRICAL CONDUCTIVITY OF METALS[1]

BY C. D. NIVEN[2]

Abstract

It is pointed out that mathematicians in their attempts to form theories of electrical conduction, do not lay emphasis on the fact that at low temperatures resistance, as a rule, does not vanish. In those cases in which it does, it vanishes suddenly. In view of this, the question arises as to whether the right model for conductivity in a metal is visualized. It is suggested that fundamentally a metallic atom is one in which the electron configuration is incomplete.

Ordinary conduction consists of a process whereby an electron jumps from one atom to another and remains with the atom it jumps to until it is in a sort of equilibrium with the motions of the electrons already on that atom. In the superconducting state, the electronic orbits of different atoms become synchronized so that when an electron leaves one atom, another electron automatically comes on to the atom to take its place. A study of the resistance-temperature curves as well as other points emphasizes the importance of structure in conductivity.

Introduction

The electrical conductivity of metals has of recent years been receiving much attention on account of the fact that existing theories do not explain experimental results. Sommerfeld, Houston and Bloch have published elaborate mathematical treatises on the problem. All of these writers assume in their treatment of the problem a free electron gas; Sommerfeld's treatise led to an expression for resistance which did not involve temperature at all, while the other two writers obtained formulas which agreed approximately with experiment, but implied that at the absolute zero of temperature the resistance would vanish. Hall has developed a dual theory in which some of the electrons are free to traverse long paths, several times the length of the mean free path, and some can pass only through the mean free path. Hall's theory evades certain difficulties but it must be admitted that no theory up to the present is entirely adequate.

There is no definite experimental evidence that at the absolute zero of temperature resistance vanishes; on the contrary, the resistance-temperature curves indicate that if resistance is to be zero at the absolute zero of temperature, the resistance will vanish suddenly as in the case of superconductors and not gradually disappear as the temperature is lowered. Again, there are certain marked peculiarities in different temperature-resistance curves which have to be accounted for. A study was made of the different temperature-resistance curves by plotting the results of experimenters and a classification was made. The curves fell roughly into three groups:— (i) Straight lines between 273 and 20° K, then curving very sharply; most divalent metals are in this group. (ii) Slightly curved all the way down; most monovalent elements lie in this group. (iii) Very curved; the common trivalent elements iron,

[1] *Manuscript received May 27, 1931.*
Contribution from the National Research Laboratories, Ottawa. This paper was read before Section III of the Royal Society of Canada, in May, 1931.
[2] *Assistant Research Physicist, National Research Laboratories, Ottawa.*

aluminium, chromium and cobalt are in this group. This classification draws attention to the importance of atomic structure in the problem of conductivity and this is still further endorsed by the recent work on alloys at very low temperatures. By alloying bismuth and gold, a superconductor has been obtained. If bismuth gives up an electron to the gold atom in much the same way as a sodium atom gives up an electron to the chlorine atom in the formation of sodium chloride, the Bi-Au electronic configuration would become the Pb-Hg one; in the same way the Bi-Tl structure could become a Pb-Pb; or the Tl-Sb structure could become a Pb-Sn. It is of interest to note that the addition of bismuth to thallium raises the superconductivity threshold temperature by 3.9° K and thereby gives a temperature very close to that of lead.

The free electron theory does not make any provision for taking into account the structure of the atom. If it were successful in deducing a formula for the variation of resistance with temperature, that formula would have to involve quantities relating either to the crystal structure or the atomic structure of the metal, in order that the formula fit all the curves, for the curves of different metals are strikingly different. The rough classification of the curves decidedly indicates that atomic structure is the important factor. This indication is supported by the fact that a single crystal does not give a very different temperature-resistance curve from an aggregate of crystals of the same metal.

The fact is that far too simple a picture of the problem is usually visualized, very questionable assumptions are made and emphasis is not laid on the points on which our knowledge is very incomplete. Furthermore the subject of conductivity extends into almost every field in physics so that as soon as a theory is constructed with elaborate care to fit the facts disclosed in one field, the new theory is completely disproved by the facts from another field. It seems probable therefore that the best way of approaching the problem is to consider first the question—what is a metal? After forming clearly some sort of a picture of a metal, the next thing to emphasize is the vital difficulties in forming a theory. As our knowledge of experimental fact is very incomplete, any theory should be sufficiently elastic so that it may be supplemented as our knowledge grows.

What Is a Metal?

A glance at a list of the elements will show that the majority of them are metals. A metal is usually a base-forming element but some metals can also be acid forming—*e.g.*, chromium in chromates and iron in ferrocyanides. The metallic atom in these last cases acts as a central atom round which the other atoms are built; incidentally the metallic atom takes on one or more electrons when it acts as the central atom in an acid, and gives up one or more electrons when it acts as a base. An essential feature therefore for an element to be a metal seems to be that it can give up one or more electrons.

Hydrogen must be held as an exception to this statement of the rule for the reason that it forms molecules which act as atoms with closed shells and therefore molecular hydrogen cannot be looked upon as capable of giving up an electron when it is in the liquid or gaseous state. From this exception we

can see how to extend our ideas of a metal and postulate that a metal is a solid or liquid substance composed of atoms or groups of atoms with the electron configuration incomplete. By a complete configuration we mean one which has two electrons of azimuthal quantum number one and six of azimuthal quantum number two in the outermost configuration. Hydrogen and helium are special cases for obvious reasons.

In order to test this definition let us consider the elements which are non-metals. The rare gases and nitrogen must, by this definition, be non-metals. According to the latest system of grouping for homopolar molecules, oxygen and probably the halogens, in their diatomic state, can be considered as having completed electronic systems. This leaves sulphur, phosphorus and selenium to be accounted for; perhaps carbon, silicon, germanium and zirconium also should not be looked upon as entirely metallic. In the case of sulphur and phosphorus it is most probable that atoms combine to form molecules. Selenium and the elements in the carbon group lie on the border between metals and non-metals. At any rate it is clear that those elements with their systems most incomplete are the most metallic and also that elements like the rare gases which have completed systems are definitely not metals. If the incomplete system is a fundamental characteristic of a metal, one can easily understand why all attempts to explain intermetallic compounds on the laws of valency were useless, for if the compounds had obeyed the laws of valency they would have lost their metallic property.

As an illustration of the formation of alloys, the Heusler alloys might be considered. The structure of iron according to the usual notation for electronic grouping is described by:– 2 : 2.6 : 2.6.6 : 2. Now this is very strongly ferromagnetic, which is in all probability due to a peculiarity in the electronic structure; therefore, if the Heusler alloys can be regarded as having a like structure, the cause of their behavior might be considered as accounted for. If Mn_3Al is largely the cause of the ferromagnetism then the explanation is simple, for by withdrawing three valence electrons from the aluminium the manganese molecule can at once be given the structure of an iron molecule (A).

$$
(2{:}2.6)\ \mathrm{Al}\begin{cases}\rightarrow \mathrm{Mn}\ (2{:}2.6{:}2.6.6{:}2)\\ \text{——}\ \mathrm{Mn}\ (2{:}2.6{:}2.6.6{:}2)\\ \rightarrow \mathrm{Mn}\ (2{:}2.6{:}2.6.6{:}2)\end{cases}
$$

(A)

The magnetic alloy Mn_4Sn can be explained by the same reasoning; the tin would give up four electrons, one of which would be received by each of the four manganese atoms. Alloys like MnB, MnAs, MnSb, MnBi probably owe their ferromagnetism to a nickel-like structure. These examples are given merely to show how combination with another element may result in a compound being formed by an interchange of electrons—the structure is not completed as in an ordinary chemical compound like a salt but a new structure is formed as in the alloys just discussed. The difficulty in ascribing a structure to a metallic compound in general is that we have not the key as in the case of these ferromagnetic compounds.

Before leaving the discussion of metals it might be of interest to point out that certain oxides and sulphides of metals are conductors and therefore

might appear to form an exception at first sight, but this probably means that the resulting electronic configuration is not what would normally be expected—a sort of alloy is probably formed.

If we accept the picture of the metallic atom that has been described above, it is possible to proceed and discuss the conductor and the electric current. In the first place, a metal appears from crystal data to have atoms closely packed together (2). The question arises at once,—how do the various atoms influence each other by their proximity?—and here we have to admit our knowledge is quite inadequate. By photographing the absorption of a rare gas in the gaseous and liquid state McLennan and Turnbull (6) have shown that the electronic configuration of a gas does not radically change when the gas is liquefied, and if we can extend this result to atoms which have incomplete systems, we can consider the metal as made up of atoms of atomic structure similar to that shown by spectroscopy. Whether this is a permissible assumption or not is uncertain but probable. Here, therefore, is the first great difficulty that confronts one in developing a theory of conduction, and until more data are at hand to explain the forces which hold atoms together in the liquid and solid states one cannot hope to form a perfect theory. For the present, we must make the assumption that the atoms in a metal have the electronic configuration indicated by spectroscopy. This, as discussed above, is an incomplete system.

The Process of Conduction

In every theory of conduction one must assume that there are conductivity electrons in the metal. The motion of these electrons as a whole constitutes the current. Now, from what has been discussed above, a metallic atom appears to be one that has the power to take on an electron or give up one, and if, as Benedicks has suggested, the metallic atoms in a conductor act as transporters of electrons, one would expect a good conductor to be composed of atoms in which the outer shell was very incomplete and therefore suitable to transport a conductivity electron. Hence one should expect good conductivity in the alkali and noble metal group.

The close connection between electro-affinity and conduction was discovered quite a number of years ago by Benedicks (1). He found that the carrying capacity of an element, *i.e.*, the atomic conductivity divided by the characteristic frequency, was related to its position in the periodic table and suggested that the carrying capacity was concerned with electro-affinity. On the assumption which Benedicks made, of the atom acting as an electron carrier, the writer attempted, in a previous paper, to reconcile Drude's treatment of the electron gas applied to electrical conduction with Benedick's idea of an atomic carrier.

Although this may be done with a fair measure of success, there is this objection: it assumes that the atoms move. If the atoms in a metal are "close-packed" this movement must be exceedingly small. When the theory was subsequently followed up, a much more serious objection was discovered in connection with the transmission and reflection of light. If we consider the process of conduction to consist of a periodic motion of the charged atoms,

backwards and forwards, we must assume some periodic frequency; Benedicks used the frequency employed in Einstein's specific heat formula. In the treatment of the problem by a method analogous to Drude's treatment, the frequency turns out to be $\frac{v}{l}$, where l is the mean free path and v is the velocity of the atoms due to heat agitation. The ratio $\frac{v}{l}$ which has the dimensions of frequency, has to be put proportional to temperature if the resistance is to be put proportional to temperature. But if we take the formula for reflection, $100-R=\frac{200}{\sqrt{CT}}$, where R is percentage of light reflected, C is the conductivity and T is the period of the light, we find that the formula holds only down to a certain wave-length. This limiting value is presumably connected with the frequency of oscillation of the atoms. Therefore, if this limiting frequency for which the reflection formula holds does not change with temperature, the frequency of oscillation of the atoms during the process of conduction must be a constant and therefore Einstein's specific heat frequency must be chosen, and not the ratio $\frac{v}{l}$. If this reasoning is correct, the question arises as to what led to the mistake of choosing $\frac{v}{l}$. It seems clear that if the rate at which the atoms are to oscillate backwards and forwards is constant, then to explain the variation of resistance with temperature we must find some way of connecting either the number of transporting atoms or the number of electrons each can carry with the temperature, and the mistake entered in not taking into consideration that the number of carriers might change with temperature.

Obviously, to solve that part of the problem, some sort of statistical method is necessary, and that belongs to the field of the mathematical physicist. But there are very important points to decide before any attempt need be made to apply mathematics. In the first place, can a carrier carry more than one electron? How does the atom act as a carrier? In all probability only one electron at a time is carried by an atom and it is reasonable to suppose that the electron describes an orbit about the nucleus of the transporting atom. Now if there were thermal agitation present, then the orbit of the additional electron would be bombarded like the others and so a sort of equilibrium would be reached. Energy would thus be used up and this would account for the development of heat in conduction. In other words it is the motion of the outermost electrons of the transporting atom that is the important factor in determining the resistance; motion of these electrons is dependent on the thermal agitation, which in turn is dependent on thermal energy.

This conception is very much reinforced by a careful examination of Gruneisen's empirical formula, $\rho=\frac{T}{\theta}F\left(\frac{T}{\theta}\right)$, where $F\left(\frac{T}{\theta}\right)$ is the specific heat function of $\left(\frac{T}{\theta}\right)$ and θ is a characteristic temperature, sometimes a little different from the Debye characteristic temperature. $F\left(\frac{T}{\theta}\right)$ can be regarded as the rate of change of energy with a change in the modulus of distribution,

while the meaning of the factor $\frac{T}{\Theta}$ is better understood by writing it $\frac{KT}{h\nu}$, in which case it is the ratio of two energies. Considering the term $\frac{T}{\Theta}$, that is to say $\frac{KT}{h\nu}$, when KT is large compared to $h\nu$, then the resistance must be large. Now KT is associated with the energy of the atoms due to thermal agitation but the meaning of $h\nu$ is not known. The most that can be said is that it is the energy connected with a frequency ν. If there is anything at all in Benedicks' conception, it would appear as if $h\nu$ were the energy liberated when the electron jumps from one atom to the next. This quantity is fixed by the atomic and lattice structure of the metal and apparently if it is insignificant compared to the thermal agitation, it is difficult for the electron to get through or, in other words, the resistance is high.

But the resistance, according to Grüneisen, is also proportional to $F\left(\frac{T}{\Theta}\right)$, *i.e.*, the rate of change of energy with change of temperature. Increase of temperature implies bombardment of the outer orbits of atoms by other atoms or molecules; the result of this bombardment is, of course, distortion of the electronic orbits if the atom cannot move and, in a "close-packed" solid, atomic movement must be small if present at all. The introduction of an additional electron to the system must also cause an increase in the distortion. Therefore in a sense the passage of an electron acts like thermal bombardment. Hence $F\left(\frac{T}{\Theta}\right)$ may be looked upon as the increase in energy per unit of disturbance caused by passage of electrons.

The Effect of a Magnetic Field on Resistance

Exhaustive research along this line has been done by Kapitza. In his theoretical treatment of the subject (5) he shows that with large fields the resistance increases in proportion to the field; at low fields there is something that prevents the effect of the field appearing. The shape of the curve indicates that the resistance increase due to increase in field strength is of a similar nature to the resistance increase due to temperature. Kapitza has apparently proved that both the temperature-resistance curve and the magneto-resistance curve indicate a residual resistance at low temperature in the one case and at low field strength in the other. The full meaning of the term residual resistance is not yet quite understood.

Nevertheless in line with the speculations of other investigators, let us for the present consider the residual resistance as due to the construction of the metal, *i.e.*, how the metallic atoms are bound together, how the orbits are arranged and how their paths interlock. This conception is endorsed by the following facts: (a) that alloying always affects the residual resistance while it often may have very little effect on the slope of the temperature-resistance curve as a whole; (b) that tempering a piece of drawn or rolled metal will as a rule merely decrease the residual resistance.

When a conductor is placed in a magnetic field, it is reasonable to assume

that the orbits of the electrons, particularly in the incompleted shell, might be turned round and oriented in space differently from the way they are when there is no field. Since we know nothing about the orientations of orbits in a metallic mass, we need not attempt to discuss the effect of a field on them. We can say however that the action of the field on the orbits of the atom is to give them additional energy. This has been proved for single atoms by the theory of the Zeeman effect and, if resistance depends on the additional electron coming into some sort of equilibrium with the motion of the outer electrons of the transporting atom, it is not unreasonable to assume that it must come into equilibrium with the increased motion given to the outer electrons of the atom by virtue of the magnetic field. If such be the case, the increase in resistance should be proportional to the magnetic field and this is just what Kapitza found for strong fields.

When the field is small however this law is not true. Kapitza showed mathematically that an assumption is necessary which supposes that there is something about the atom that prevents the action of the field taking effect. With low fields this action predominates, while with strong fields it is negligible. If we extend the ideas which have been explained above, an explanation can be offered for this hypothesis of Kapitza's, for while the energy is undoubtedly given to the electrons, there is also a re-orientating of the orbits. These orbits themselves, before the field was put on, had a field which had an accelerating action on the electron, and the electron had to get into a state of equilibrium with this field: by being turned in their orientation they lost so much of that influence on the additional electron. The net effect therefore with a weak field was accordingly slight. Not until all the twist possible was given these orbits would the effect on the additional electron be proportional to the field strength. This suggests that in the atom itself there is electron motion, possibly resulting in a magnetic field with which the additional electron must come into equilibrium. This would suggest a residual resistance. When the field is put on, the orbits of the electrons are turned sufficiently to destroy this motion or "internal field", and leave merely the external magnetic field itself with which the additional electron must come into equilibrium. After the maximum amount of twist possible has taken place the resistance increase is proportional to the field strength.

Superconductivity

The residual resistance found by Kapitza's work and the residual resistance found by investigating the resistance-temperature curves at low temperature are, according to Kapitza, the same. The suggestion outlined above would require this. Now, since there is a small thermal motion even at 4° K as well as the electron motion, which for lack of a better name we have called the "internal field", it is obvious that the phenomenon of superconductivity cannot be accounted for unless it be no longer necessary for this equilibrium to be taken up by the additional electron. The logical conclusion is that there is no additional electron at all in the superconducting state. It would seem as

though instead of an additional electron jumping from atom to atom, in the superconducting state one of the electrons from one atom must jump to the next just at the moment when an electron from that atom has left. Such a process would imply a synchronizing of all the orbits. This synchronizing would of course be easily upset by the bombardment, due to thermal agitation, and one would also expect that a magnetic field would as a rule upset it. At the same time one would expect that it might be possible to orient the orbits in such a way that the synchronized state was more easily reached. This may account for the fact that adding bismuth to a superconductor (7) seems to raise the superconducting threshold temperature. One would rather expect that pressure would raise the superconducting threshold temperature. This experiment has not been performed and might present experimental difficulties.

The analogy of a set of gears might be used to explain the writer's idea in regard to the difference between the superconducting and the normal conducting state. In the superconducting state everything is timed like a set of gears in a mesh; in the normal state every atom acts as a gear, running separately or at least meshing only part of the time.

The explanation suggested above for the difference between superconductivity and normal conductivity gives a ready explanation of why at the superconducting temperature there is no discontinuity in the variation with temperature of specific heat, thermal conductivity, crystal structure or other physical property: for there is no difference in the structure and arrangement of the atoms—the electrons have merely got into an ideally synchronized arrangement.

Conclusion

The evidence shows that structure is of the highest importance in the problem of electrical conductivity. The term "free electron" implies that the bond between the so-called "free" electron and the nucleus is to be ignored. Any theory based on such an assumption can of course only be valid when the thermal agitation is so violent that the force exerted by this bond is insignificant compared with the force on the free electron when a neighboring electron strikes it. Grüneisen's formula as shown by Borelius (3) is in far better agreement with experiment than formulas given by the modern free electron theories, and it is hoped that this discussion will at any rate serve the purpose of emphasizing the importance of the Benedicks-Grüneisen viewpoint of conduction.

References

1. BENEDICKS, C. Jahrb. Radioakt. Elektronik, 13: 351. 1916.
2. BERNAL, J. D. Trans. Faraday Soc. 25: 367-379. 1929.
3. BORELIUS, G. Z. Physik, 54: 806-810. 1929.
4. GRÜNEISEN, E. Ber. physik. Ges. 20: 53-62. 1918.
5. KAPITZA, P. Proc. Roy. Soc. Lond. 123A: 292-372. 1929.
6. MCLENNAN, J. C. and TURNBULL, R. Proc. Roy. Soc. Lond. 129A: 266-283. 1930.
7. MCLENNAN, J. C., ALLEN, J. F. and WILHELM, J. O. Trans. Roy. Soc. Can. 24, III: 53-61. 1930.

FROST PRECIPITATION OF PROTEINS OF PLANT JUICE[1]

By R. Newton[2] and W. R. Brown[3]

Abstract

An exposure of 5 hr. at −7° C. caused maximum precipitation of the proteins of press-juice from leaves of unhardened winter wheat grown in the greenhouse. The value of sucrose added as a protection against precipitation increased with concentration up to about 8%. This concentration of total sugar is often attained by hardy varieties of winter wheat in the field. Sucrose and dextrose were about equally effective. By microscopic examination of smears caused by drops of juice, to which various quantities of dilute acid or base had been added, flowing across glass slides, it was ascertained that maximum precipitation occurred at about pH 5.1 and 7.3, respectively. Added sugar reduced the percentage precipitation by acid, base or "salting out." The addition of acid or common salt, insufficient to cause any immediate effect microscopically visible, increased subsequent frost precipitation; the addition of base or alkaline buffers decreased it. The removal of natural electrolytes by dialysis increased subsequent frost precipitation. Added sugar stabilized the juice proteins under all conditions. Precipitation by frost, acid or base are all irreversible. The coagula produced by different agencies acting on the juice have distinctive characteristics, which are illustrated. It is concluded that dehydration is the basic cause of frost precipitation, and that ice formation, acidity, salt concentration, and possibly pressure, are all contributing factors.

Introduction

Frost precipitation of the proteins of plant juice can easily be demonstrated, and has been thought to be the immediate cause of the frost-killing of plant tissue. While the exact mechanism of the precipitation has been in doubt, it has been clear that the withdrawal of water from the cells by ice formation in the intercellular spaces leads to an increased salt concentration and acidity of the sap. Either of these factors might bring about precipitation, and both have been suggested (2, 3, 12) as the agency.

On the other hand, there are factors which tend to stabilize the protoplasm, making it more resistant to disorganization by contact with concentrated sap. Two, chiefly, have been emphasized in the literature. The accumulation of sugars in the sap (4, 5, 12) and the splitting of the proteins to simpler, less readily precipitated forms (3, 12), during the hardening process, have been regarded as protective influences.

Protein-splitting has been investigated to some extent by the present authors (7, 8, 9) and will receive further attention in a later paper. Sugar accumulation has also been reported on, in the papers just cited, with the general conclusion that it tends to be greater during the winter in hardy varieties. This relation has been demonstrated by a number of workers, most convincingly by the long-continued investigations of Åkerman (1). Preliminary experiments already published (8) on the protection afforded by the sugars gave results in harmony with the views of other workers cited above.

The present paper reports a series of experiments done in 1924-25, on the frost precipitation of the proteins in the press-juice of winter wheat plants, as

[1] *Manuscript received June 8, 1931.*
Contribution from the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada.
[2] *Professor of Field Crops and Plant Biochemistry, University of Alberta.*
[3] *Formerly postgraduate student, University of Alberta.*

affected by salt concentration, acidity and sugar content, and by intensity and duration of exposure. By these experiments *in vitro*, it was hoped to gain a better understanding of the mechanism of precipitation and of the significance to be attached to the sugar concentrations naturally occurring in the field, as a protective adaptation of hardy plants.

Materials and Methods

Since it was desired to investigate the various factors affecting precipitation and the protective action of sugars, the press-juice of greenhouse plants was used (except in one or two specified cases) in order to obtain protein colloids in an unhardened condition and unprotected by any appreciable natural concentration of sugar. We have shown elsewhere (9) that proteins constitute the bulk of the colloidal material in the press-juice of wheat leaves, and that sugars in the juice of unhardened plants run less than 0.5% concentration. Two varieties of winter wheat were chiefly used: Minhardi, a hardy variety, and Fulcaster, a non-hardy variety. Two others were used in a few experiments: Buffum, a hardy variety, and Kanred, which is semi-hardy.

In sampling, the plants were cut off just above ground level. The material thus obtained consisted of leaves, since in no case had jointing of the plants taken place. The press-juice was prepared and handled with all possible precautions to prevent changes in the constituents other than those induced experimentally. The method of extracting the juice has already been described in detail (10).

Greenhouses controlled with regard to light and temperature were not available at the time of these experiments, and unavoidable fluctuations in these and other environmental conditions of the plants were no doubt responsible for much of the variability in the results. The plant material used in the various experiments is in most cases described in the tables with regard to the date of sampling, the age of the plants from date of seeding, and the content of total and coagulable nitrogen in the press-juice. The date affected the light and temperature conditions in the greenhouse, the temperature in winter running around 70° F. and in summer paralleling roughly the outdoor temperature, but at a higher level, especially on sunny days. The age of the plants affected the sensitivity of the proteins, and the concentration of nitrogen in the juice was found by Schaffnit (12) to affect the percentage of precipitation. The experiments in any one series were carried out simultaneously with portions of the same lot of juice.

Test tubes of 25 cc. capacity, with rubber stoppers, were used as containers for the juice during exposure. The freezing baths of ice slush and salt were well insulated and of large capacity. Where, however, the temperature of the bath changed appreciably during the period of exposure, the temperatures at the beginning and end are both stated in the tables. In the experiments on sugar protection, a tube of pure juice, another of juice with added sucrose, and in some cases a third tube containing juice with added dextrose were placed together in a freezing bath. When different concentrations of sugar were

being tested at a given temperature, the necessary number of tubes were exposed together in one bath. In the tables, the number of grams of sugar added to 25 cc. of juice has in each case been multiplied by 4 and recorded as a per cent.

After exposure, the samples of any one group were thawed in running water, and immediately whirled in a centrifuge for a standard time (10 min.). The supernatant fluid was decanted, and two samples of 10 cc. pipetted out for the determination of nitrogen. The values found were corrected where necessary to allow for changes in volume on the addition of sugar or other reagents. The percentage of nitrogen precipitated by the exposure was found by comparison of the total nitrogen in the supernatant fluid with the total and heat-coagulable nitrogen determined in the fresh press-juice.

The methods for determining nitrogen have already been described (9). The Kjeldahl method used for total nitrogen was not modified to include nitrates, and it was discovered that the added sugar reduced the nitrates and occasionally led to finding more nitrogen in the sugar-protected samples after freezing and centrifuging than in the original fresh juice. This difficulty had not been encountered with juice from field-grown plants, which contained sufficient natural sugar to reduce the nitrates. It was corrected in the latter part of the work with juice from greenhouse plants by equalizing all samples with respect to sugar content just before the Kjeldahl digestion.

The concentration of sugar usually employed in experiments involving sugar-protection, except when the effect of concentration was the point at issue, was either 8 or 4%. The former concentration is often reached naturally by the juice of field-grown plants in the late fall, and the latter concentration approaches the minimum found in hardy varieties at that time of the year (7, 8, 9). Both sucrose and dextrose were used in the first experiments, both being naturally present in considerable quantity in hardened wheat plants, and both being effective in reducing frost precipitation (8).

Effect of Temperature of Exposure on Frost Precipitation

The first experiments were designed to ascertain the effect of the temperature of exposure on the degree of precipitation, with and without sugar protection. Six series, lettered A to F, are recorded in Table I.

The experiments of Series A disclosed the surprising result that less precipitation occurred at the lower temperatures than at the higher ones. It was thought possible that at the lower temperatures complete congelation took place so rapidly that there was not time for dehydration of the colloidal particles to proceed to such a damaging extent as where the freezing took place more slowly. To offset this possibility, in Series B, C and D, the temperatures were lowered in steps, by transferring the tubes to progressively colder baths at intervals of an hour. For example, in the fifth experiment of Series B, the three tubes were first placed in a bath at $-4\frac{1}{2}$° C. for an hour, then transferred to a bath at -6° C. for the second hour, then to a bath at -12° C. for the third hour, and finally to a bath at -21° C. for $2\frac{1}{2}$ additional

hours, making a total exposure of 5½ hours. This method of exposure, however, did not alter the trend of the results to more than a slight degree, and an explanation must wait on further investigation.

The results of Series A to D show, with one minor exception, the greatest precipitation in the unprotected juice to take place at −7° C. This coincides with the first point of absolute killing of the tissue of both varieties used. The latter was determined by placing in test tubes fresh plants corresponding to those from which the juice was obtained, and immersing one tube in every freezing bath used, for the same length of exposure as the juice. At −3° C.

TABLE I

EFFECT OF TEMPERATURE OF EXPOSURE ON FROST PRECIPITATION, WITH AND WITHOUT SUGAR PROTECTION

Material	Exposure		Sample	Per cent total N pptd.	Per cent coag. N pptd.
	Time, hr.	Temp., ° C.			
A. Minhardi	9	− 3	Juice only	36.9	56.7
Feb. 27, 1924,					
plants 44 days old.	8	− 5 to −4½	Juice only	47.9	73.8
N in 10 cc. juice:			Juice+8% S*	40.0	61.6
total, 53.7 mg.			Juice+8% D*	40.7	62.6
coag., 34.9 mg.					
	8	− 8½ to −7	Juice only	50.6	77.9
			Juice+8% S	18.4	27.4
			Juice+8% D	19.0	29.2
	7	−11½	Juice only	37.4	57.6
			Juice+8% S	20.5	31.6
			Juice+8% D	24.2	37.2
	5	−21	Juice only	20.7	31.8
			Juice+8% S	6.9	10.6
			Juice+8% D	7.0	10.8
B. Fulcaster	5	− 3	Juice only	28.6	46.9
Feb. 29, 1924,			Juice+8% S	12.7	20.9
plants 46 days old.			Juice+8% D	12.3	20.2
N in 10 cc. juice:					
total, 40.5 mg.	5	− 4½	Juice only	48.0	78.7
coag., 24.7 mg.			Juice+8% S	33.0	54.2
			Juice+8% D	30.2	49.5
	1	− 4½	Juice only	56.0	70.6
	4	− 5 to −7	Juice+8% S	30.5	50.1
			Juice+8% D	31.3	51.4
	1	− 4½	Juice only	43.0	70.6
	1	− 6	Juice+8% S	19.0	31.2
	3½	−12 to −10	Juice+8% D	26.4	43.3
	1	− 4½	Juice only	36.1	59.3
	1	− 6	Juice+8% S	11.9	19.6
	1	−12	Juice+8% D	13.2	21.7
	2½	−21			
	5½	−21	Juice only	25.3	41.5
			Juice+8% S	6.9	11.3
			Juice+8% D	5.8	9.6

**S = sucrose; D = dextrose.*

TABLE I—*Continued*

Material	Exposure: Time, hr.	Exposure: Temp., ° C.	Sample	Per cent total N pptd.	Per cent coag. N pptd.
C. Minhardi	5	0	Juice only	0.4	0.7
Mar. 5, 1924,			Juice+4% D	0.0	0.0
plants 51 days old.					
N in 10 cc. juice:	5	− 4½ to −3	Juice only	4.9	7.5
total, 47.4 mg.			Juice+4% S	0.0	0.0
coag., 30.8 mg.			Juice+4% D	0.0	0.0
	1	− 4½	Juice only	16.2	24.8
	4	− 8 to −7	Juice+4% S	0.0	0.0
			Juice+4% D	0.0	0.0
	1	− 4½	Juice only	4.9	7.5
	1	− 8	Juice+4% D	0.0	0.0
	3½	−12			
	1	− 4½	Juice only	1.6	2.5
	1	− 8	Juice+4% D	0.0	0.0
	1	−12			
	2½	−21			
D. Fulcaster	5	0	Juice only	1.7	2.6
Mar. 7, 1924,			Juice+4% S	1.6	2.4
plants 53 days old.			Juice+4% D	0.5	0.7
N in 10 cc. juice:					
total, 36.6 mg.	5	− 4½ to −4	Juice only	21.2	33.1
coag., 23.4 mg.			Juice+4% S	1.1	1.8
			Juice+4% D	2.2	3.4
	1	− 4½	Juice only	25.6	40.0
	4	− 7½ to −7	Juice+4% S	4.5	7.1
			Juice+4% D	1.4	2.1
	1	− 4½	Juice only	11.3	17.6
	1	− 7½	Juice+4% D	0.3	0.5
	3½	−12			
	1	− 4½	Juice only	15.8	24.6
	1	− 7½	Juice+4% S	6.2	9.7
	1	−12	Juice+4% D	2.8	4.3
	2½	−21			
E. Fulcaster	5½	0	Juice only	13.6	20.9
May 8, 1924,					
plants 62 days old.	5	− 7	Juice only	13.5	20.7
N in 10 cc. juice:			Juice+8% S	0.5	0.7
total, 28.9 mg.					
coag., 18.8 mg.					
F. Minhardi	5	3	Juice only	16.7	29.7
May 13, 1924,			Juice+8% S	6.2	11.1
plants 69 days old.					
N in 10 cc. juice:	5	0	Juice only	26.1	46.6
total, 54.1 mg.			Juice+8% S	10.7	19.1
coag., 30.4 mg.					
	5	− 7	Juice only	30.6	54.4
			Juice+8% S	7.9	14.1

the plants of Minhardi were uninjured, but those of Fulcaster showed considerable damage; at $-4\frac{1}{2}°$ C. no injury to Minhardi was yet apparent, while Fulcaster was very seriously injured; at $-7°$ C. and all lower temperatures the plants of both varieties were killed outright. It is noteworthy that even under greenhouse conditions, which are not conducive to "hardening-off", the Minhardi plants retained some of their superiority in frost resistance. In this instance, it is reflected in the relative precipitation in the unprotected juice of the two varieties, but in other experiments this was not found to be a necessary relationship.

The experiments of Series C and D were carried out a week later than those of Series A and B, with plants from the same seeding, and apparently some change had taken place in the plant material in the interval, since the precipitations in C and D were all of a much smaller order of magnitude. Such a rapid change in the apparent sensitivity to frost of the cell proteins cannot be fully explained at present, though the sensitivity does appear to decrease with age, as will be shown later.

Series A and B were carried out with an added sugar concentration of 8%; Series C and D with 4%. At first glance it appears that the smaller concentration was actually a more effective protection than the higher concentration. In view of the altered reactions of the pure juice, however, such a conclusion is not justifiable. It is clear that the value of sugars cannot be stated in absolute terms of percentage protection, but only in relative terms, and that comparisons of the value of different concentrations of sugar or of different degrees or lengths of exposure must be based on experiments with a single lot of press-juice.

Sucrose and dextrose in the present experiments appeared to be of about equal protective value, variations of small magnitude occurring in both directions. To simplify subsequent experiments, therefore, only sucrose was used. For the same reason, the temperature of $-7°$ C. was adopted for all frost exposures, since it had been found most effective in precipitating the proteins.

The short Series E and F added at the end of Table I illustrate the unexpectedly high precipitation found occasionally in juice stored at 0° C. There would appear to be some effect of cold which operates injuriously even in the absence of ice formation. Potentiometric determinations of hydrogen ion concentration, made on other samples of juice at a later date, did not show any measurable change during 5 hr. storage at 0° C. A surprising feature in this instance is that as much precipitation occurred in the Fulcaster juice at 0° C. as at $-7°$ C., while in the Minhardi juice the difference in precipitation at these two temperatures was not large. The possibility of explaining the precipitation at 0° C. on the basis of enzyme activity seems to be precluded by the fact that less precipitation occurred in Minhardi juice stored at $+3°$ C. In passing it might be noted that this precipitation at 0° C. was always in greater evidence in the juice of older plants, and that added sugar always stabilized the juice considerably, whether frozen or not.

Effect of Length of Exposure on Frost Precipitation

Having found −7° C. an effective precipitating temperature, the next step was to determine the influence of length of exposure. It seemed possible that changes induced by frost might at first be reversible but in time become irreversible, and that this might have an important relation both to precipitation of the proteins *in vitro* and to frost-killing in the field. Four series of experiments on this point are reported in Table II. To simplify the records, the per cent coagulable protein precipitated has been omitted from this and later tables. While frost precipitation was probably restricted to nitrogen

TABLE II

EFFECT OF LENGTH OF EXPOSURE AT −7° C. ON PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE

Material	Hours exposure	Per cent nitrogen pptd.	
		Juice only	Juice+8% sucrose
A. Fulcaster May 8, 1924, plants 62 days old. N in 10 cc. juice: total, 28.9 mg. coag., 18.8 mg.	1 2 3 4 5 6	4.4 8.0 9.1 8.2 13.5 12.6	4.1 4.3 1.7 0.0 0.5 1.7
B. Minhardi May 13, 1924, plants 69 days old. N in 10 cc. juice: total, 54.1 mg. coag., 30.4 mg.	1 2 3 4 5 6	7.6 10.3 17.5 26 9 30.6 37.8	0.8 1.1 1.6 4.0 7.9 24.4
C. Minhardi June 15, 1925, plants 16 days old. N in 10 cc. juice: total, 58.2 mg. coag., 39.2 mg.	1 2 3 4 5 6 7	8.2 25.5 40.4 44.7 51.3 55.6 —	0.1 0.5 1.8 2.5 1.1 2.3 5.0
D. Fulcaster July 3, 1925, plants 16 days old. N in 10 cc. juice: total, 37.0 mg. coag., 20.1 mg.	1 2 3 4 5 6 7	26 6 35.0 50.3 48.7 51.7 58.8 55.8	3 1 4.1 7.2 1.5 5.4 3.8 6.1
E. Minhardi, March 19, 1925, mixed plants 30 and 50 days old. N in 10 cc. juice: total, 64.3 mg. coag., 44.4 mg.	24	16.5	9.3
F. Fulcaster March 19, 1925, mixed plants 30 and 50 days old. N in 10 cc. juice: total, 49.2 mg. coag., 32.5 mg.	24	47.4	35.0

in the colloidal state, and the coagulable protein should therefore have more significance than the total nitrogen, the per cent precipitation of the former may readily be calculated, if desired, from the data now included in the tables.

Series A and B in Table II should be moderately comparable, since the plants used were of about the same age and grown under the same conditions. The juice of Fulcaster, the non-hardy variety, was considerably more dilute than that of Minhardi, a common distinction between these varieties, no doubt connected with their relative frost hardiness which tends to express itself under all conditions. In this instance, the Fulcaster juice appeared very stable, and might be thought to support Schaffnit's finding that precipitation is directly related to concentration of protein (12). But our own experiments do not permit any such generalization. It is contradicted in fact by the results of Series C – D and E – F in the same table. It has already been suggested that at least until such time as the experimental plants can be produced under standard conditions, controlled in all important respects, each lot of press-juice must be regarded as largely a law unto itself.

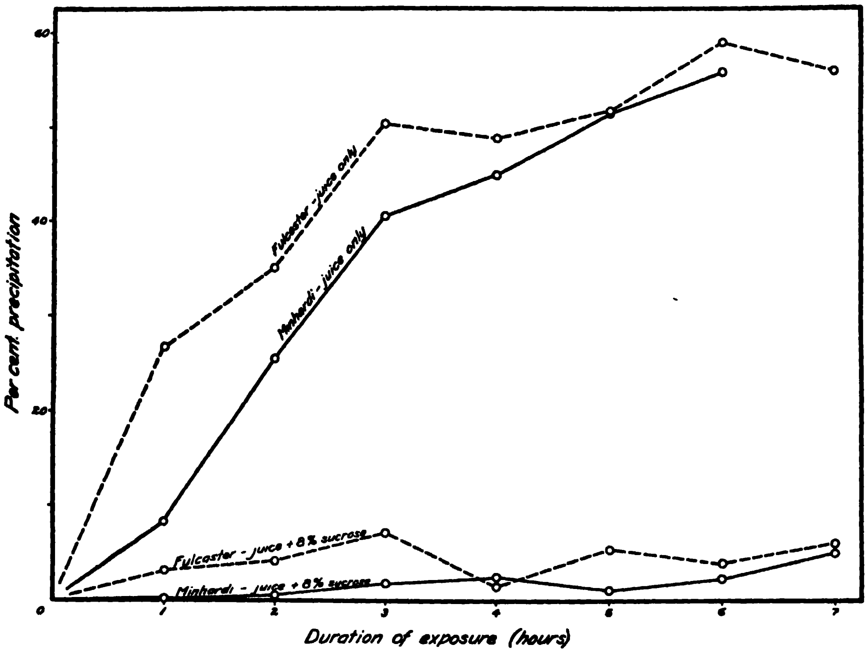


FIG. 1. *Precipitation of nitrogen in unprotected and sugar-protected juice at −7° C., in relation to length of exposure.*

For Series A, B and C, control plants in test tubes were immersed in the freezing baths with the test tubes of juice. The leaves of Fulcaster in Series A survived 2 hr. at −7°C., after which they were killed. Leaves of Minhardi in Series B showed only slight injury after 5 hr. exposure; in Series C they were killed in 2 hr. Comparing Series A and B, there is obviously no correspondence between the sensitivity of the leaves to frost-killing and of the juice to frost precipitation. But such comparison is scarcely justifiable, for the reason

given in the last paragraph. While great caution is necessary in comparing any two lots of press-juice, it is seen in Series B and C, in which only one variety comes in question, that frost precipitation is greater in the juice from the more sensitive leaves. When examination is confined to the results of one collection of one variety, either in Table I or Table II, there is found to be a direct relation between injury to the leaves and precipitation of the proteins, provided (Table I) the temperature does not go below $-7°$ C.

There is some indication in Table II that the protective value of sugar may fall off with time. This is most pronounced in Series B, and is suggested again by the single cases of long exposure listed in Series E and F. Series C and D were done at a later date, after it had been found by special experiments that the younger plants were better suited to our purpose, and the results are rather more regular and consistent. They are presented graphically in Fig. 1, in which it is seen that precipitation increased rapidly with length of exposure up to 3 hr., and after that more slowly. The protective effect of the sugar is also clearly seen.

The early experiments were made the basis of using 5 or 6 hr. as a suitable length of exposure in most subsequent experiments.

Effect of Sugar Concentration on Frost Precipitation

The sugar concentrations used in the first experiments were based on those found in the juice of winter-hardened plants in the field. It seemed desirable to examine more particularly the significance of concentration by comparisons over a wider range. Experiments to this end are reported in Table III.

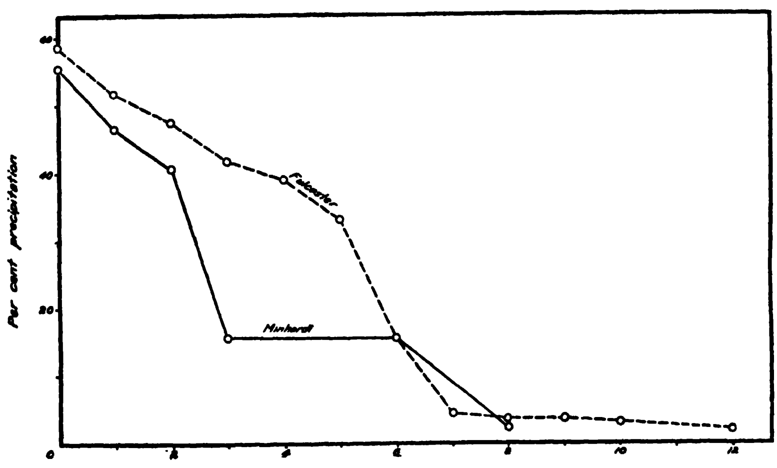


FIG. 2. *Precipitation of press-juice nitrogen in 6 hr. at $-7°$ C., in relation to added concentration of sucrose.*

Portions of the same lots of press-juice used for the experiments reported in Table II were used for those in Table III. Attention has already been directed to the characteristic difference in nitrogen concentration of the juices of the two wheat varieties, and to the association of greater stability with a

more dilute condition in Series A-B but not in Series C-D. Regardless of these differences in other properties, all juices reacted in much the same way to the addition of sugar in various concentrations. Protection increased with concentration up to 8%. This is shown again in Fig. 2, plotted from the results of Series C-D.

TABLE III

EFFECT OF ADDED SUGAR CONCENTRATION ON PRECIPITATION AT −7° C.

Material	Hours exposure	Per cent sucrose added	Per cent nitrogen pptd.
A. Fulcaster	5	0	13.5
May 8, 1924,	5	2	7.9
plants 62 days old.	5	4	2.3
N in 10 cc. juice:	5	6	1.1
total, 28.9 mg.	5	8	0.5
coag., 18.8 mg.	5	10	0.6
B. Minhardi	5	0	30.6
May 13, 1924,	5	2	30.0
plants 69 days old.	5	4	17.8
N in 10 cc. juice:	5	6	17.4
total, 54.1 mg.	5	8	7.9
coag., 30.4 gm.	5	10	8.9
C. Minhardi	6	0	55.6
June 15, 1925,	6	1	46.5
plants 16 days old.	6	2	40 5
N in 10 cc. juice:	6	3	15.7
total, 58.2 mg.	6	6	15.7
coag., 39.2 mg.	6	8	2 3
D. Fulcaster	6	0	58.8
July 3, 1925,	6	1	51.9
plants 16 days old.	6	2	47.4
N in 10 cc. juice:	6	3	41.6
total, 37.0 mg.	6	4	39.0
coag., 20.1 mg.	6	5	33.0
	6	6	15.5
	6	7	4.6
	6	8	3.8
	6	9	3.8
	6	10	3.1
	6	12	2.0

Just why the increase in sugar protection should cease rather abruptly at 8% is not clear, unless there be a small proportion of the protein of a nature not amenable to stabilizing by sugar. This seems unlikely, since in some other experiments (Table I) apparently complete protection was obtained. It might be thought that higher concentrations would perhaps exercise a dehydrating influence on the proteins, especially since the effective concentration would be very much increased by the crystallizing of water on freezing the juice. This suggestion again seems untenable, since we have found (Table IX) that sugar reduces "salting out", when its effect should be additive rather than inhibitive if it acted as a dehydrating agent. It is significant, in any case, to find that the concentration required to give maximum protection against protein precipitation is not greater than that often attained by hardy varieties of winter wheat in the field.

Effect of Age and Condition of Plants

Because of the variability in press-juice properties encountered in the early experiments, some of the factors which might be responsible were investigated, particularly the effect of age of plants. The juice of field-grown plants usually increases in concentration of both total solids and nitrogen with age (9) while that of greenhouse plants, within the limits of age observed in these experiments, does not do so. The greenhouse juice is generally more dilute than the field juice, at any stage, but fluctuates up and down with temperature and moisture conditions. It might be supposed that if conditions were held constant the properties of the juice would also remain constant. There is some evidence, however, that the sensitivity of the proteins to frost decreases with the age of the plants.

TABLE IV

EFFECT OF AGE OF PLANTS ON PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE

Variety and exposure	Date	Age in days	Mg. N in 10 cc. juice		Per cent nitrogen pptd.	
			Total	Coag.	Juice only	Juice + 8% sucrose
Minhardi Exposed 5 hr. at −7° C.	Mar. 2	13	47.1	—	35.6	—
	Feb. 13	16	44.1	29.1	56.3	0.0
	Mar. 9	20	54.3	--	48.7	0.2
	Feb. 20	23	44.9	23.3	42.2	0.0
	May 13	69	54.1	30.4	30.6	7.9
Fulcaster Exposed 5 hr. at −7° C.	Mar. 4	15	40.9	25.4	48.3	—
	Feb. 13	16	38.5	25.0	54.1	0.0
	Feb. 20	23	40.3	22.5	52.8	0.0
	May 8	62	28.9	18.8	13.5	0.5
Minhardi Exposed 5 hr. at 0° C.	Feb. 13	16	44.1	29.1	0.0	0.0
	Feb. 20	23	44.9	23.3	3.1	0.0
	May 13	69	54.1	30.4	26.1	10.7
Fulcaster Exposed 5 hr. at 0° C.	Feb. 13	16	38.5	25.0	0.3	0.0
	Feb. 20	23	40.3	22.5	5.7	0.0
	May 8	62	28.9	18.8	13.6	—

In Table IV are gathered the results of a number of experiments on precipitation at −7° C. and at 0° C., the data for each temperature and variety being arranged in order of age of plants. The collections of February 13 and 20 were made from one seeding, those of March 2, 4 and 9 from another seeding, and those of May 8 and 13 from still another. The diversity of growth conditions in the greenhouse at these various times is reflected to some extent in the fluctuating nitrogen concentration in the juice. This however shows no consistent relation to the degree of precipitation, which seems rather to be related to the age of the plants. The juice proteins show a tendency to become less sensitive to frost but, on the other hand, less stable at 0° C., as the plants advance in age.

To reduce the variability of the results, the practice was adopted of using as far as possible only young plants, in a state of full turgor at the time of cutting. The turgidity affects of course the concentration of the juice.

Effect of Adding Acid, Base, and Salt on Precipitation without Freezing

It seemed that the possible role of acidity and salt concentration in frost precipitation could in certain aspects be investigated best by experiments on the effect of these factors on unfrozen juice, and on the relation of sugar to their activity. Harvey (3) titrated with $N/10$ H_2SO_4 and $N/10$ NaOH, cabbage juice obtained from the midribs and from the rest of the leaves, having an original pH of about 5.8 and 5.6 respectively. He reported instantaneous precipitation with acid at about pH 5.1 in the midrib juice and pH 4.3 in the other juice, and with alkali at pH 6.6 in both juices. All of these points lie on the acid side of neutrality. The range between the precipitation points he concluded was the optimum hydrogen ion concentration for holding the proteins in solution in the sap. Since the increased hydrogen ion concentration required to precipitate the proteins by titration was closely paralleled by that which he found in the fluid portion of juice precipitated by freezing, when the ice crystals were removed, he concluded further that the increased acidity was the active agent in both cases, and that the increased salt concentration on freezing was insufficient of itself to cause precipitation.

Titrating wheat plant juice with dilute sulphuric acid and sodium hydroxide, we found the precipitation took place gradually over a range of about one pH unit, and to determine the maximum point developed the following technique. To a definite volume of juice in a test tube kept in an ice bath at 0° C., the reagent was added in definite increments. With 20 cc. of juice the increment of reagent was usually 0.5 cc. After each addition the juice was thoroughly stirred with a glass rod, and one drop allowed to run across a glass microscope slide fixed at an angle of 45°. A microscopic examination of the series of smears thus obtained made it possible to determine the point of maximum coagulation. The corresponding hydrogen ion concentration was determined upon another portion of juice to which was added a volume of reagent equal to that which brought the first portion to the point of maximum precipitation. All determinations of hydrogen ion concentration were made with a Leeds and Northrup type K potentiometer and a bubbling hydrogen electrode.

TABLE V

Maximum precipitation points at 0° C. found by titration with acid and base

Variety	Date 1925	Age in days	Mg. N in 10 cc. juice		Reagent added to 20 cc. juice		pH of juice	
			Total	Coag.	Kind	Cc. required	Original	At max. pptn.
Minhardi	Mar. 2	13	47.1	—	$N/5$ H_2SO_4	2.5	5.95	5.02
					$N/5$ NaOH	2.5	5.95	7.28
Fulcaster	Mar. 4	15	40.9	25.4	$N/5$ H_2SO_4	2.0	5.98	5.10
					$N/5$ CH_3COOH	3.0	5.98	5.02
Fulcaster	Mar. 13	24	—	—	$N/5$ H_2SO_4	2.5	6.00	5.16
					$N/5$ CH_3COOH	3.5	6.00	5.03

The results of some experiments of this kind are given in Table V. They indicate the precipitation points to lie at about pH 5.1 and pH 7.3. These can be considered only as approximate, since the acid and base were added in 0.5 cc. increments. More precise titrations may show the points of maximum precipitation with acid, which now appear similar for both varieties of wheat, to be identical. The precipitation occurs in stages corresponding to the hydrogen ion concentration. A series of smears representing an increasing number of additions of acid shows first a granulation which grows by steps to a distinct coagulum. After the maximum precipitation point is passed, the coagulated particles begin to disperse, and chemical action begins to show itself in the form of discoloration of the juice. Five stages in this process are shown in Fig. 4, *a-e*, made by tracing circular portions, 1 mm. in diameter, projected on a glass screen by a micro-projector.

When dilute sodium hydroxide is added to the juice, the precipitate is usually indistinct and always much less general than that caused by acid. In one experiment, dilute ammonium hydroxide was tried, but caused no precipitation. An interesting point seen in the experiment with Minhardi juice (Table V) is that it took the same volume of $N/5$ acid or base to bring about precipitation. Since the hydrogen ion concentration of the juice was not halfway between the two precipitation points, but nearer to the acid one, it would appear that it was more strongly buffered against acid than against base.

It is also of interest to note that of the two collections of Fulcaster, the one with older plants yielded a more strongly buffered juice, requiring more sulphuric or acetic acid to bring about the same change in pH, than the juice of the younger plants. This may have a bearing on the greater sensitivity to frost of the juice proteins of the younger plants noted in the preceding section.

The results of the titration experiments do not support Harvey's conclusion that the whole zone between the precipitation points represents optimum conditions for solution of the proteins, with instantaneous precipitation marking the limits. A relatively slight change in the hydrogen ion concentration of wheat juice, caused by adding acid or alkali, is sufficient to induce granulation which, however small, indicates incipient precipitation.

TABLE VI

HYDROGEN ION CONCENTRATION OF PRESS-JUICE OF FIELD-GROWN PLANTS AT DIFFERENT AGES

Variety	Date of collection	Age in days	pH
Kanred	June 22	13	5.60
Seeded June 9, 1925	June 29	20	5.34
	July 6	27	5.31
	July 13	34	5.38
	July 20	41	5.32
	July 27	48	5.00
	Aug. 3	55	4.87

The precipitation points found in wheat juice do not coincide with those found by Harvey in cabbage juice, except in the case of the cabbage midrib juice, which precipitated at about pH 5.1. This is not surprising, since we have found that even wheat plants must vary in their precipitation points when grown under different conditions. In Table VI are given the results of a series of determinations of hydrogen ion concentration of field-grown plants of Kanred wheat at progressive ages. If the properties of the press-juice of these plants had been the same as those of the greenhouse plants used in the experiments of Table V, it is clear that the juice of the last two collections should have precipitated spontaneously, whereas nothing of the sort occurred. Whether the isoelectric points of the proteins were actually different, or whether their stability was attributable to other factors, cannot now be stated.

Using the above method of finding the point of maximum precipitation, a number of quantitative experiments were made on the amount of precipitation caused by titration of the juice held at 0° C. The juice was centrifuged after titration, the nitrogen determined in the supernatant fluid, and the per cent precipitation found by difference. The results are presented in Table VII.

TABLE VII

MAXIMUM PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE AT 0° C. FOUND BY TITRATION WITH $N/5$ H_2SO_4 AND $N/5$ NaOH

Variety	Date 1925	Age in days	Mg. N in 10 cc. juice		Reagent	Per cent nitrogen pptd.	
			Total	Coag.		Juice only	Juice+8% sucrose
Minhardi	Feb. 13	16	44.1	29.1	Acid Base	63.3 21.6	48.3 —
Minhardi	Feb. 20	23	44.9	25.3	Acid Base	61.5 7.8	53.7 0.0
Fulcaster	Feb. 20	23	40.3	22.5	Acid	64.9	47.9

The per cent nitrogen precipitated by acid was closely similar in the three cases, amounting to nearly two-thirds of the whole. A calculation will show that this represented about 109% of the coagulable protein in the second case and 116% in the third case. Evidently some of the non-coagulable proteins were carried down in the acid precipitate. The precipitation by the base was very much less, suggesting that the bulk of the proteins had their isoelectric point on the acid side. The addition of sugar afforded significant protection against the action of both acid and base. This apparently was not because of any effect on the reaction of the mixture, since a few determinations of the pH showed in all cases that of the pure juice and of the juice plus sucrose to be identical, even after the samples had stood for two days.

A modified form of experiment was carried out, in which the juice was not titrated but had added to it definite quantities of dilute acid or base, after

which it was allowed to stand 6 hr. at 0° C. It will be remembered that in earlier experiments the proteins had shown a considerable tendency to precipitate at this temperature (Table IV). The results given in Table VIII, and

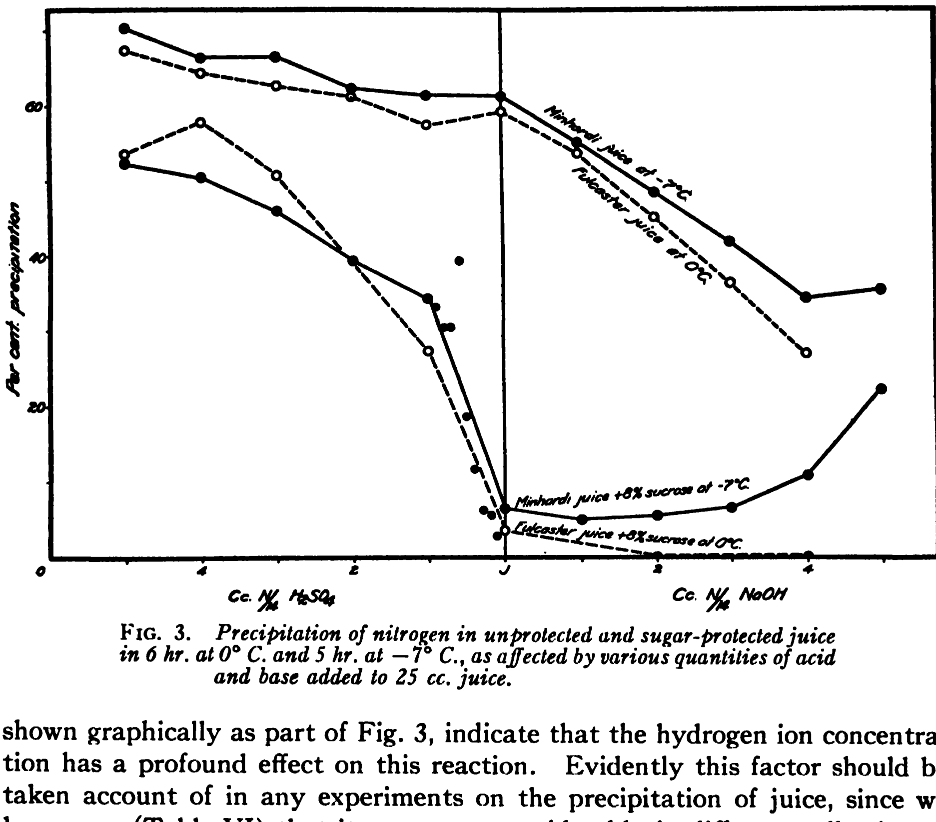


FIG. 3. *Precipitation of nitrogen in unprotected and sugar-protected juice in 6 hr. at 0° C. and 5 hr. at −7° C., as affected by various quantities of acid and base added to 25 cc. juice.*

shown graphically as part of Fig. 3, indicate that the hydrogen ion concentration has a profound effect on this reaction. Evidently this factor should be taken account of in any experiments on the precipitation of juice, since we have seen (Table VI) that it may vary considerably in different collections.

TABLE VIII

EFFECT OF ADDING ACID AND BASE ON PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE IN 6 HR. AT 0° C.

Material	Reagent added to 25 cc. juice		Per cent nitrogen pptd.	
	Kind	Vol. in cc.	Juice only	Juice+8% sucrose
Fulcaster	$N/14$ H_2SO_4	5	67.3	53.4
June 17, 1925,	$N/14$ H_2SO_4	4	64.3	57.8
plants 16 days old.	$N/14$ H_2SO_4	3	62.7	50.8
N in 10 cc. juice:	$N/14$ H_2SO_4	2	61.2	—
total, 51.3 mg.	$N/14$ H_2SO_4	1	57.2	27.4
coag., 34.9 mg.	—	0	59.3	3.4
	$N/14$ NaOH	1	53.8	—
	$N/14$ NaOH	2	45.1	0.0
	$N/14$ NaOH	3	36.5	—
	$N/14$ NaOH	4	27.0	0.0

The change in hydrogen ion concentration of the naturally buffered juice caused by the small amounts of $N/14$ acid and base added was not sufficient to bring about any immediate granulation which could be observed microscopically. This may be judged in the case of the acid by comparison with Fig. 4, *a*. In the course of 6 hr., however, the acid increased precipitation while the base decreased it. The increments of acid had less effect on the unprotected juice than those of base, possibly because the juice was more strongly buffered against acid than against base, as already noted, but more probably because the original juice was already within the margin of its precipitation zone and in the liberal time allowed for precipitation the acceleration of the reaction by added acid became relatively unimportant. The sugar-protected juice, however, was so stable that the quantity of acid added became very important. The combination of sugar and dilute alkali prevented all precipitation.

TABLE IX

SUGAR PROTECTION AGAINST "SALTING OUT" OF JUICE SATURATED WITH ZINC SULPHATE AT 0° C.

Variety	Date 1925	Age in days	Mg. N in 10 cc. juice		Per cent nitrogen pptd.	
			Total	Coag.	Juice only	Juice+8% sucrose
Buffum	Aug. 24	19	40.8	18.8	58.4	48.9
Kanred	Aug. 24	19	54.6	25.5	82.1	70.3

Salt concentration, the other factor commonly supposed to be active in frost precipitation, was investigated in one experiment at 0° C. with regard to its relation to sugar protection. The results presented in Table IX show that here again the sugar is effective at least to some extent in stabilizing the proteins and reducing precipitation.

Effect of Adding Acid, Base and Salts on Frost Precipitation

If increased acidity and salt concentration on freezing are important factors responsible for frost precipitation of plant proteins, then it would be expected that the addition of acid or salt to plant juice before exposure to frost should increase the amount of precipitation, while the addition of base should decrease it. That this is the case is shown by the experimental results collected in Table X, and for Series C recorded graphically as part of Fig. 3. The small quantities of the reagents added produced no coagulation before freezing which could be observed microscopically. Their concentration would of course be increased many times by the withdrawal of water as ice. The reaction would probably move in the acid direction on freezing, even in those samples to which base had been added, the system being dominated by the natural acids of the juice.

The effect of acid was the same in all experiments in which the concentration added was sufficient to have any effect at all. It is most easily seen in the results with Minhardi juice in Series C, which have been incorporated in

TABLE X

EFFECT OF ADDING ACID, BASE AND SALTS ON PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE IN 5 HR. AT $-7°$ C.*

Material	Reagent added to 25 cc. juice		Per cent nitrogen pptd.	
	Kind	Quantity	Juice only	Juice + 8% sucrose†
A. Fulcaster March 7, 1924, plants 53 days old. N in 10 cc. juice: total, 36.6 mg. coag., 23.4 mg.	*N*/14 HCl *N*/14 NaOH	1 cc. 1 cc.	12.1 0.0	— —
B. Minhardi May 13, 1924, plants 69 days old. N in 10 cc. juice: total, 54.1 mg. coag., 30.4 mg.	*N*/14 H_2SO_4 *N*/14 H_2SO_4 None *N*/14 NaOH *N*/14 NaOH NaCl	2 cc. 1 cc. — 2 cc. 3 cc. 0.5 gm.	41.2 38.8 30.5 27.9 23.9 44.6	— — — — — —
C. Minhardi July 1, 1925, plants 16 days old. N in 10 cc. juice: total, 31.8 mg. coag., 15.4 mg.	*N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 *N*/14 H_2SO_4 None *N*/14 NaOH *N*/14 NaOH *N*/14 NaOH *N*/14 NaOH *N*/14 NaOH	5.0 cc. 4.0 cc. 3.0 cc. 2.0 cc. 1.0 cc. 0.9 cc. 0.8 cc. 0.7 cc. 0.6 cc. 0.5 cc. 0.4 cc. 0.3 cc. 0.2 cc. 0.1 cc. — 1.0 cc. 2.0 cc. 3.0 cc. 4.0 cc. 5.0 cc.	70.1 66.4 66.5 62.3 61.3 — — — — — — — — — 61.3 55.3 48.6 41.9 34.6 35.5	52.3 50.6 46.0 39.4 34.3 33.4 30.5 30.6 39.2 18.8 11.6 6.1 5.7 2.8 6.5 5.0 5.5 6.6 10.8 22.2
D. Minhardi March 2, 1925, plants 13 days old. N in 10 cc. juice: total, 47.1 mg.	None NaCl	— 0.25 gm.	35.6 47.4	— 7.7
Fulcaster March 4, 1925, plants 15 days old. N in 10 cc. juice: total, 40.9 mg. coag., 25.4 mg.	None NaCl	— 0.25 gm.	48.3 52.7	— 6.7

In Series A, exposure was 4 hr. at 0°C. followed by 2 hr. at $-21°$ C

†*In Series F, concentration of sucrose was 4%.*

TABLE X—*Continued*

Material	Reagent added to 25 cc. juice		Per cent nitrogen pptd.	
	Kind	Quantity	Juice only	Juice+8% sucrose†
E. Buffum	None	—	65.6	—
Aug. 24, 1925,	$N/1$ Na_2HPO_4	1 cc.	62.7	16.8
plants 19 days old.	$N/1$ NaCl	1 cc.	60.1	15.8
N in 10 cc. juice:	$N/1$ CH_3COONa	1 cc.	59.9	10.5
total, 40.8 mg.	$N/1$ Na_2CO_3	1 cc.	39.5	12.1
coag., 18.8 mg.				
Kanred	None	—	59.1	—
Aug. 24, 1925,	$N/1$ Na_2HPO_4	1 cc.	59.5	13.1
plants 19 days old.	$N/1$ NaCl	1 cc.	56.3	27.3
N in 10 cc. juice:	$N/1$ CH_3COONa	1 cc.	52.1	13.7
total 54.6 mg.	$N/1$ N_2CO_3	1 cc.	27.2	16.8
coag., 25.5 mg.				
F. Kanred	None	—	43.4	36.0
July 21, 1924,				
plants 21 days old.	$N/14$ H_2SO_4	1 cc.	41.4	39.9
N in 10 cc. juice:				
total, 48.4 mg.	NaCl	0.25 gm.	41.5	35.5
coag., 27.5 mg.				
Juice as above,	None	—	80.0	22.8
dialyzed.				
N in 10 cc:	$N/14$ H_2SO_4	2.5 cc.	92.0	—
total, 20.5 mg.				
coag., 18.1 mg.				
Kanred	None	—	73.0	17.3
July 25, 1925,				
plants 25 days old,	$N/14$ H_2SO_4	0.8 cc.	87.8	86.7
juice dialyzed.				
N in 10 cc.:	NaCl	0.25 gm.	63.4	44.0
total, 13.9 mg.	NaCl	0.50 gm.	51.8	—
coag., 11.5 mg.	NaCl	0.75 gm.	45.5	—

**In Series A, exposure was 4 hr. at 0°C. followed by 2 hr. at −21° C.*
†In Series F, concentration of sucrose was 4%.

Fig. 3. They follow very closely the results with Fulcaster juice obtained without freezing, which are included in the same figure and have been discussed above. There is this difference, however, that in the Minhardi samples the addition of base was carried to a higher concentration, and shows signs of having gone beyond the limit of the zone in which its action is protective.

Experiments on the effect of adding common salt occur in Series B, D, E and and F. The last series will be discussed below. In the other three, it will be seen that when the added concentration was 1% (0.25 gm. to 25 cc.) or over, the frost precipitation was increased. In Series E, three other salts, with buffer properties, were added in equimolar concentrations. The percentage thus varied with the molecular weights, being in the case of the common salt about 0.23%. This was probably too small to exercise any "salting out" effect, even when concentrated by freezing. In effect on precipitation, the

four salts fell in the same order with both Buffum and Kanred juice, and the order was definitely related to their buffer properties. The acid sodium phosphate reduced the precipitation only slightly if at all, while the sodium carbonate, an alkaline buffer, reduced it very greatly.

Series F includes three experiments, in the last two of which the juice was dialyzed free from electrolytes. The purpose was to examine separately the salting-out and acid-precipitation effects, uncomplicated by the action of acids and salts naturally present in the juice. Two features of the results are especially noteworthy.

First, the precipitation of the dialyzed juice was greater than that of the original juice, when no sugar had been added to either. The comparison here is best made on the basis of coagulable protein, since the non-coagulable nitrogen was largely removed by dialysis. In Kanred collected July 21, the frost precipitation was equal to 76% of the coagulable protein in the original juice and 90% in the dialyzed juice. In the collection of July 25, the precipitation was equal to 88% of the same fraction in the dialyzed juice. Thus it appears that the practically complete removal of natural electrolytes increased frost precipitation, and we must perhaps ascribe the whole of the precipitation in the dialyzed juice to the direct dehydration of the proteins by abstraction of water as ice.

The addition of a small amount of dilute acid to the dialyzed juice increased precipitation to more than the equivalent of the total coagulable protein. This was observed before, in titrating fresh juice with acid and alkali (Table VII), when it was concluded that some of the non-coagulable proteins were precipitated by acid.

The second striking feature of the experiments of Series F is the protection against frost precipitation of dialyzed juice afforded by added salt. This increased with concentration within the 1 to 3% range used. Such a result was unexpected at the time, though it was encountered again later (11), when it was found that the removal of crystalloids from plant-juice by dialysis made the proteins very unstable, and that their stability could be partly though not completely preserved by dialyzing against a 1% solution of common salt.

Taking Table X as a whole, no more significant generalization emerges than the value of sugar in stabilizing the proteins of the juice under all experimental conditions. When the reagent, like dilute acid, is precipitative, sugar is inhibitive; when the reagent, like dilute alkali, alkaline buffers, or in the dialyzed juice, salt, is protective, the action of the sugar is additive.

Irreversibility of Precipitation and Types of Coagulum

The degree of irreversibility of the precipitation caused by frost or added reagents *in vitro* may perhaps be taken as some indication of the probable destructiveness of the same kind of reaction in the living plant. A few simple experiments were done to see whether the various coagula could be redispersed.

A tube of Minhardi juice was exposed to $-7°$ C. for 5 hr. The juice was then thawed and shaken vigorously for several minutes. Microscopic examination

KEY TO FIG. 4

Minhardi juice
First visible granulation
20 cc. juice
7 cc. $N/14$ H_2SO_4

a

Minhardi juice
Flocculation
20 cc. juice
12 cc. $N/14$ H_2SO_4

b

Minhardi juice
Maximum precipitation
20 cc. juice
15 cc. $N/14$ H_2SO_4

c

Minhardi juice
Beginning of dispersion
20 cc. juice
17 cc. $N/14$ H_2SO_4

d

Minhardi juice
Increasing dispersion
20 cc. juice
30 cc. $N/14$ H_2SO_4

e

Minhardi juice
After neutralization
20 cc. juice
2.5 cc. $N/5$ H_2SO_4
2.5 cc. $N/5$ NaOH

f

Fulcaster juice
Maximum precipitation
20 cc. juice
2 cc. $N/5$ H_2SO_4

g

Fulcaster juice
After neutralization
20 cc. juice
2 cc. $N/5$ H_2SO_4
2 cc. $N/5$ NaOH

h

Fulcaster juice
Sugar-protected
20 cc. juice
1.6 gm. sucrose
2 cc. $N/5$ H_2SO_4

i

Minhardi juice
Frost precipitation
5 hr. at $-7°$ C.

j

Minhardi juice
Sugar-protected
5 hr. at $-7°$ C.

k

Minhardi juice
Heat coagulation
30 min. at 97.5° C.

l

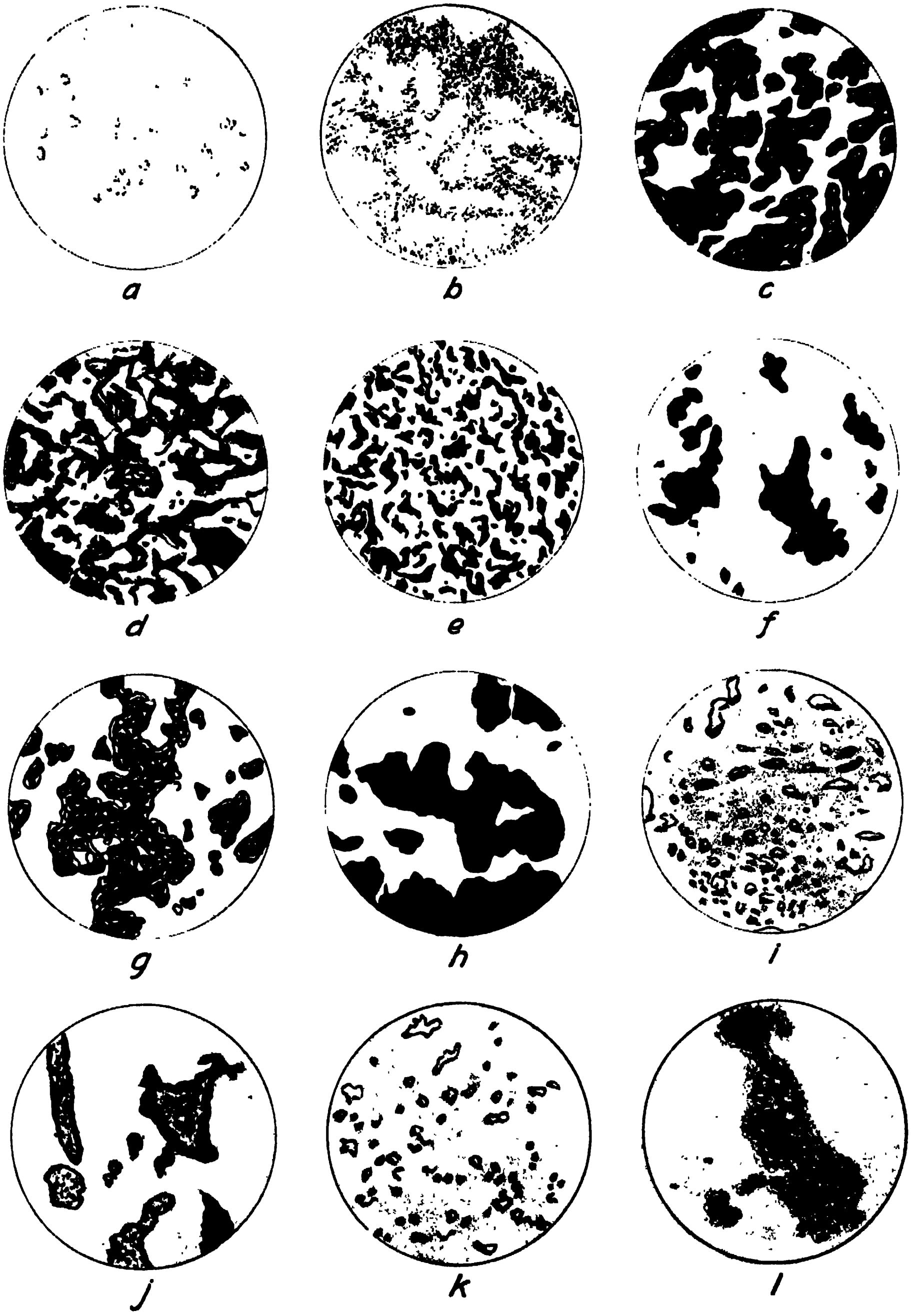


FIG. 4. *Tracings of micro-projections of 1-mm. portions of smears caused by drops of juice flowing across glass slides held at an angle of 45°.*

of the juice before and after shaking showed that instead of redissolving, the small particles of coagulum had flocculated into larger masses, the effect being analogous to the formation of butter by churning. That this shaking even incorporated some of the finer particles which would otherwise have remained in suspension upon centrifuging, is shown by the fact that the supernatant fluid from shaken juice contained 0.8 mg. of nitrogen less per 10 cc. than that of the unshaken control sample.

When small quantities of acid or base were added to wheat-leaf juice a precipitate was obtained when the acidity of the solution reached about pH 5.1 or pH 7.3 respectively. In order to see whether this precipitation was reversible, samples were neutralized by the addition of an equivalent quantity of base or acid. Instead, however, of dissolving the particles this procedure invariably resulted in the formation of a more pronounced, denser precipitate. There seemed to be a further abstraction of water from the particles, causing them to shrink and take on the appearance of a firmer texture. This behavior is illustrated in Fig. 4, *c* and *f*, *g* and *h*. That this denser precipitate was not due to the salt formed by the chemical combination of the acid and base, was shown by the lack of any trace of precipitate when an amount of neutral salt solution, equal to that formed by the combination of the two reagents, was added to the juice. In fact when double this amount of salt was added no precipitation occurred.

When the smear method of examining the precipitation points of juice protein was first used, it was noted that the coagula produced by various agencies were very different.

At the acid precipitation point, the particles of the coagulum formed are always granular in structure and honeycombed with holes (Fig. 4, *c* and *g*). They are light green in color and very irregular in outline. If this coagulum is neutralized by a base, the particles immediately become more compact and regular in outline and take on a denser appearance (Fig. 4, *f* and *h*).

At the basic precipitation point the coagulated particles are dark green in color and, unlike the large, irregular, granular particles of the acid coagulum, they are small, somewhat spherical in shape, and of a more even-textured appearance. If acid is added these spherical particles at once aggregate and form a coagulum similar to that of the neutralized acid coagulum.

If sugar is first added to the juice and then acid, a coagulum is formed which is very similar in general character to, but with larger and more numerous particles than, the basic coagulum just discussed (Fig. 4, *i*). If sugar is added, then a base, the particles are scarcely visible.

The particles of the coagulum formed by heat are large, granular in structure but almost colorless (Fig. 4, *l*). In general shape and size they resemble the acid-coagulated particles, but are a little more compact than the latter.

Unlike any of the coagula so far described, that formed by exposure to frost appears to consist of thin flakes, often roughly rectangular in outline, and generally rolling up at the edges (Fig. 4, *j*). The structure of the flakes is homogeneous rather than granular. The sugar-protected frozen juice shows a character similar to that of the basic coagulum (Fig. 4, *k*).

Another feature noticed during the course of the work was that the heat coagulum of unprotected juice which had just been frozen, contains white curds to the extent of about a fourth of its volume. This is observed only when heat coagulation is preceded by exposure to frost, and suggests that freezing brings about a separation of some of the pigments and proteins.

Discussion

The nature of the experiments described in this paper precludes a high degree of accuracy in the results. Plants grown under uncontrolled conditions are highly variable in their properties, and some of the laboratory manipulations required are not amenable to strict precision. For example, precipitation was not always clean cut, and before sampling it was necessary to decant the whole of the fluid portion after centrifuging, since pipetting from various depths in the test tube would yield fluid of different concentrations. There is, nevertheless, sufficient consistency in the results to justify certain deductions.

It seems clear that both "salting out" and acid precipitation play a part in frost precipitation, and that sugar is an important factor in protecting the protoplasmic proteins from disorganization by these agencies. It cannot be concluded, however, that salt and acid are the fundamental factors in precipitation, since this took place to an even greater extent in dialyzed juice, and under these conditions the addition of a small concentration of salt exerted a protective influence.

It seems probable that dehydration of the proteins is the basic cause of precipitation, and that the agency is incidental rather than fundamental. The degrees of precipitation brought about by freezing, by titration with acid, and by saturation with zinc sulphate, were in general quite similar, and may simply indicate that all these agencies, when given full scope, are about equally effective in dehydrating the protein particles. The withdrawal of water as ice may well be in ordinary circumstances the dominating factor, but the experimental results leave no doubt that acidity and salt concentration are important modifying influences.

The possible effect of the direct pressure developed in the system by freezing must not be overlooked. Maximov (6), in his recent review of the principal theories concerning frost-killing of plant tissue, reaffirms the view he first put forward in 1914, that the reason is the mechanical injury of the protoplasm caused by the compression of the cells by ice crystals which accumulate in the intercellular spaces. That protoplasm may be coagulated by pressure is well known to physiologists. The very labile cell proteins dispersed in press-juice would undoubtedly be subjected to pressure when the system was frozen. Under this influence the frost coagulum might take on the flaky structure which distinguished it from all others, much as the gluten in bread dough takes on a honeycomb structure under the influence of internal gas pressure. Pressure may denature the proteins, and contribute to the irreversibility of the precipitation.

The term precipitation has been used in this paper in the general sense of

the separation of the solid and liquid phases. When the separation takes place at the isoelectric point, as in titration with acid, initially it may be true precipitation, but it is evidently followed by denaturation, since the process is irreversible. Salting out may follow somewhat the same course. When heat is the agent, undoubtedly the process is coagulation. The true character of frost precipitation may be open to question. That which results from dehydration by ice crystallization would seem to be properly classified as coagulation, being irreversible, and must be the condition in dialyzed juice. In ordinary circumstances, frost precipitation may occur at the isoelectric point, and may then be a combination of true precipitation and true coagulation.

The mechanism of sugar protection is still obscure. Maximov (6), after reviewing the work of various authors, concludes that the possibility of a purely chemical activity of sugars is not excluded, but that the action is more probably physico-chemical, based on the molar concentration of the sugar, that is, on the number of molecules present in the solution. He further affirms that the main point in this activity is the retention of part of the water in an unfrozen state, since he believes that it is the quantity of ice formed during freezing which conditions the death of plant cells.

Our results have produced objections to both of Maximov's conclusions. If the activity is proportional to molar concentration, this must be true only within fairly restricted limits, since approximately maximum protection is afforded by 8% of sucrose. On this basis, maximum protection with dextrose should be reached at a little over 4%. The results in Table I, Series D, do show in three cases out of four a slight superiority of 4% dextrose over 4% sucrose. This lends color to the view that at that concentration dextrose is exerting its maximum protective activity while sucrose is not. The point should be examined in further experiments. Maximov's belief that the activity of sugar depends chiefly on the retention of part of the water in an unfrozen state, meets a difficulty in the fact that protection is equally demonstrated when no ice at all is formed, as when the juice is precipitated by titration or salting out, or simply allowed to precipitate spontaneously on standing at 0° C.

The added sugar had no measurable effect on the hydrogen ion concentration of the juice, as influenced by standing at 0° C. or by freezing and thawing. It is true that we did not press out the fluid from the ice crystals for separate measurements, as Harvey did (3), and we could not determine the pH after the juice solidified at about −2° C. But under conditions which precipitated unprotected juice without freezing, the sugar-protected samples at the same pH did not precipitate.

Sucrose in high concentrations, such as should result when an 8% solution freezes, is usually regarded as a dehydrating agent, and might be expected to increase salting out. Sucrose and dextrose theoretically should differ in this respect, the former being hydrated in solution and the latter probably not. Comparative experiments on this point have not been carried out with the two sugars, but we have seen that when sucrose was added to the juice, the amount of salting out was not increased but, on the contrary, decreased.

Some chemical effect of added sugar will be indicated in the next paper of this series, but a complete explanation of sugar protection must await further investigation. It seems possible that additional light may be found in the colloidal phenomena of adsorption and viscosity.

References

1. ÅKERMAN, Å. Studien über den Kältetod und die Kälteresistenz der Pflanzen nebst Untersuchungen über die Winterfestigkeit des Weizens. Lund. 1927.
2. GORKE, H. Über chemische Vorgänge beim Erfrieren der Pflanzen. Landw. Vers. Stat. 65: 149-160. 1906.
3. HARVEY, R. B. Hardening processes in plants and developments from frost injury. J. Agr. Research, 15: 83-112. 1918.
4. LIDFORSS, B. Die wintergrüne Flora. Lunds Univ. Arsskr. 2, No. 13. 1907.
5. MAXIMOV, N. A. Chemische Schutzmittel der Pflanzen gegen Erfrieren. Ber. deut. botan. Ges. 30: 52-65, 293-305, 504-516. 1912.
6. MAXIMOV, N. A. Internal factors of frost and drought resistance in plants. Protoplasma, 7: 259-291. 1929.
7. NEWTON, R. A comparative study of winter wheat varieties with especial reference to winter-killing. J. Agr. Sci. 12: 1-19. 1922.
8. NEWTON, R. The nature and practical measurement of frost resistance in winter wheat. Univ. of Alberta, Coll. of Agr., Research, Bull. 1. 1924.
9. NEWTON, R. and BROWN, W. R. Seasonal changes in the composition of winter wheat plants, in relation to frost resistance. J. Agr. Sci. 16: 522-538. 1926.
10. NEWTON, R., BROWN, W. R. and MARTIN, W. M. The extraction of plant tissue fluids and their utility in physiological studies. Plant Physiol. 1: 57-65. 1926.
11. NEWTON, R. and MARTIN, W. M. Physico-chemical studies on the nature of drought resistance in crop plants. Can. J. Research, 3: 336-427. 1930.
12. SCHAFFNIT, E. Studien über den Einfluss niederer Temperaturen auf die pflanzliche Zelle. Mitt. Kaiser-Wilhelm Inst. Landw. Bromberg, 3: 93-144. 1910.

STUDIES IN THE VARIABILITY OF TUBERCLE BACILLI. II. CORRELATION OF COLONY STRUCTURE, ACID AGGLUTINATION AND VIRULENCE[1]

By Guilford B. Reed[2] and Christine E. Rice[3]

Abstract

Twenty-eight cultures of tubercle bacilli including human, bovine and avian forms from widely differing sources have been compared as to colony structure, habit of growth in fluid media, acid agglutination and virulence for animals.

It was found that the recently isolated highly virulent cultures and all the cultures with a long history of high virulence grew on solid media in S colony form; on fluid media, in the case of bovine and human types, as a continuous veil-like pellicle, in the avian types as a diffuse suspension; all were agglutinated only at a high acidity. All the avirulent or low virulent cultures or those with a history of loss of virulence now grow on solid media in R colonies; in fluid media as a heavy pellicle tending to separate into discrete islands; and are agglutinated at a relatively low acidity.

In the first paper in this series, Reed and Rice (20), it was shown that under appropriate cultural conditions a rapid-growing strain of bovine tubercle bacilli might be separated, on the basis of colony structure, into two types, R and S, which exhibit some degree of stability or true breeding qualities. Under similar conditions several other acid-fast species, particularly *Mycob. leprae* and *Mycob. phlei* (19) were separated into two definite types, R and S, and several intermediate types. These results were shown to be in conformity with the earlier work of Petroff and associates on the tubercle bacilli.

Petroff (13, 14, 15, 16) demonstrated that certain cultures of human, bovine and avian tubercle bacilli may be dissociated by suitable cultural procedure into R and S colony types. A culture of avian organisms which he had had under cultivation for many years was dissociated into an S type, highly virulent for chickens, and an avirulent R type. The differentiation of these types has since been confirmed by Kahn and Schwarzkopf (6) and additional distinguishing characteristics added. The Saranac strain of human bacilli, H37, which has frequently been used in experimental work, generally characterized by high virulence for guinea pigs, has occasionally failed to produce progressive disease, a condition which Petroff has shown in certain instances to be the result of dissociation from S to R. A bovine strain which had long been under cultivation was successfully dissociated into a virulent S type and an avirulent R type. In the same manner Petroff has separated R and S types from several cultures of B.C.G.

As a further phase of a general study of these phenomena a considerable group of cultures of tubercle bacilli have been examined as to colony structure,

[1] *Manuscript received May 29, 1931.*

Contribution from the Department of Bacteriology, Queen's University, Kingston. This work has been carried on with the co-operation and the financial assistance of the Associate Committee on Tuberculosis of the National Research Council. This paper forms one section of a thesis submitted by Dr. Rice to the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] *Professor of Bacteriology, Queen's University.*

[3] *Research Fellow in Bacteriology, Queen's University.*

acid agglutination and virulence for laboratory animals. In this particular investigation we have not been concerned primarily with the experimental production or observation of the progress of dissociation but with examination and correlation of these three characteristics in a considerable group of cultures. For the most part these are strains which have been under cultivation for a long time and several are cultures which have been extensively used in experimental studies of tuberculosis.

The results reported in this paper are largely based upon a study of colonies appearing on the first series of plate cultures made from the original tubes taken from our own culture collection or as received from other collections. Petroff's gentian violet-egg was used as a plating medium. This was made according to Petroff's (12) original formula except that only one-half the specified gentian violet was added. The reaction was accurately adjusted with the aid of the hydrogen electrode to pH 7.8 before sterilization and the sterilization was carried out at 100° C. In some instances Petri dishes were used, sealed with paraffin except for a small air vent; in the majority of cases Petroff's excellent modification of Kolle flasks, now made in Pyrex glass, were used. These permit examination of colonies with a 25 to 50-diameters binocular microscope almost as readily as when the organisms are grown in Petri plates.

As a further measure of the characteristics of the several cultures, suspensions were subjected to acid agglutination. In this procedure the growth was removed from the surface of the media with a spatula, ground in either a mortar or a small ball mill*, with the addition of distilled water, drop by drop, to form an even suspension. After the organisms had been washed three times by centrifuging in distilled water, the washed suspension was added to a series of phosphate buffer solutions ranging from pH 2.0 to 5.0. The resulting agglutination was read after incubation for two hours at 40° C. As has been observed in many such studies, agglutination occurred at a particular pH zone. In the brief statement of results with many cultures, in this paper, the highest pH to produce a definite agglutination is taken as the end-point. In the case of a culture which is completely agglutinated from pH 2.0 to 3.2 the latter figure is recorded as the agglutination point.

Acid agglutination was resorted to as a means of differentiation in one of the earliest studies of bacterial dissociation; DeKruif (3) separated the organisms of rabbit septicemia into two types on the basis of colony structure and demonstrated a correlation between this characteristic and both virulence for animals and the point of acid agglutination. Since this work of DeKruif's much has been done on the potential difference of bacteria in many aspects. Most significant in this connection is the demonstration of Kahn and

This small ball mill has been found very useful in preparing suspensions of tubercle bacilli for various purposes. Ordinary 125-cc. Florence Pyrex flasks into which half a dozen glass beads or better, 5-mm. steel balls as used in bearings, are added, constitute the grinding chamber. This is clamped to the centre of a horizontally revolving wheel and turned at a rate of one to ten revolutions per second. A number of these flasks are prepared, plugged with cotton, and sterilized. As required the dry pellicle or other growth mass is added, the flask clamped to the wheel and grinding commenced. Fluid may be added with a pipette as required to give an even suspension. It is no more efficient as a grinder than a mortar but it does reduce contamination to a minimum.

Schwarzkopf (6) that Petroff's R and S strains of avian tubercle bacilli show a substantial difference in electrophoretic potential. It is quite possible that we should have been better advised to follow such a practice, or McCutcheon, Mudd, Strumie and Lucké's (9) procedure of determining isoelectric points, rather than to determine the probably similar characteristics of the organisms by acid agglutination. Later and more detailed studies with a small number of cultures bear out the suggestion. However it will be observed that a definite correlation was found in a considerable group of cultures between colony structure, virulence for animals and acid agglutination. The method appears therefore to have been sufficiently accurate for the distinctions which are made in this paper.

Human Types

Human—H37

A culture of the Saranac Lake strain of virulent human type was sent to us by Dr. Petroff. The first culture on gentian violet-egg media plates produced only characteristic S colonies. Young cultures on Proskauer and Beck's fluid grew as a thin continuous veil which gradually thickened as the culture matured. Suspensions were agglutinated at pH 3.2. This apparently represents the characteristic S, virulent, human type.

Human—Strains Freshly Isolated from Sputum

Six strains have recently been isolated from the sputum of active pulmonary cases. The initial cultures consisted entirely, or largely, of definite S types. Morning samples of sputum were mixed with equal volumes of three per cent sodium hydroxide and allowed to stand with occasional shaking for two hours. Hydrochloric acid was then added to the first yellow tinge of phenol red, about pH 7.2. After centrifuging 15-cc. portions of the fluid, the supernatent 12 to 14 cc. was removed and discarded and Petroff's gentian violet-egg media inoculated from the surface of the remaining 1 to 3 cc. of fluid in such a way as to avoid the packed sediment at the bottom of the centrifuge tubes.

The initial cultures prepared in this manner and incubated for six to eight weeks exhibited very similar S colony types. Plate I, 1 to 6, are photomicrographs made from these cultures and indicate the type of colony found in the six samples. The colonies, with their granular surfaces, raised central areas sloping gradually to a very thin margin and irregular outline, closely resemble the S type of the H37 culture just described. In those cases where a good growth was obtained on a number of plates, so that thirty to fifty thousand colonies could be compared, a few R-like types were found. A study of these types from a number of typical and atypical cases is now in progress and will be reported on in the near future.

All these strains with the S colony structure showed acid agglutination at approximately the same pH level and in sharp contrast to the R human types mentioned in this paper. The results are shown in Table I. The same six strains freshly isolated from sputum produced progressive tuberculosis in guinea pigs.

TABLE I

SUMMARY OF THE COLONY STRUCTURE, ACID AGGLUTINATION AND VIRULENCE OF A GROUP OF 28 CULTURES OF TUBERCLE BACILLI

Culture	Colony structure	Acid agglutination, pH	Virulence
Human			
H 37	S	3.2	+
Sputum 1	S	3.0	+
2	S	3.0	+
3	S	3.2	+
4	S	3.2	+
5	S	3.0	+
Koch	S	3.2	+
H 13 S	S	3.3	+
H 13 R	R	3.9	−
Ceylon	R	3.9	−
Koch-Raw	R	3.8	−
54 S	S	3.2	?
54 R	R	4.2	−
Upt	R	?	−
Bovine			
Vallée S	S	2.8	+
Vallée R	R	3.8	?
56	S	2.8	+
Calmette-Raw	R	4.2	−
Brown	R	4.0	−
599	R		−
Avian			
Petroff S	S	2.8	+
Petroff R	R	3.6	−
823	S	2.8	+
807	S	3.4	+
116	R	3.8	−
118	R	4.0	−
Bang	R—S	4.0	−
120	R—S	4.0	−

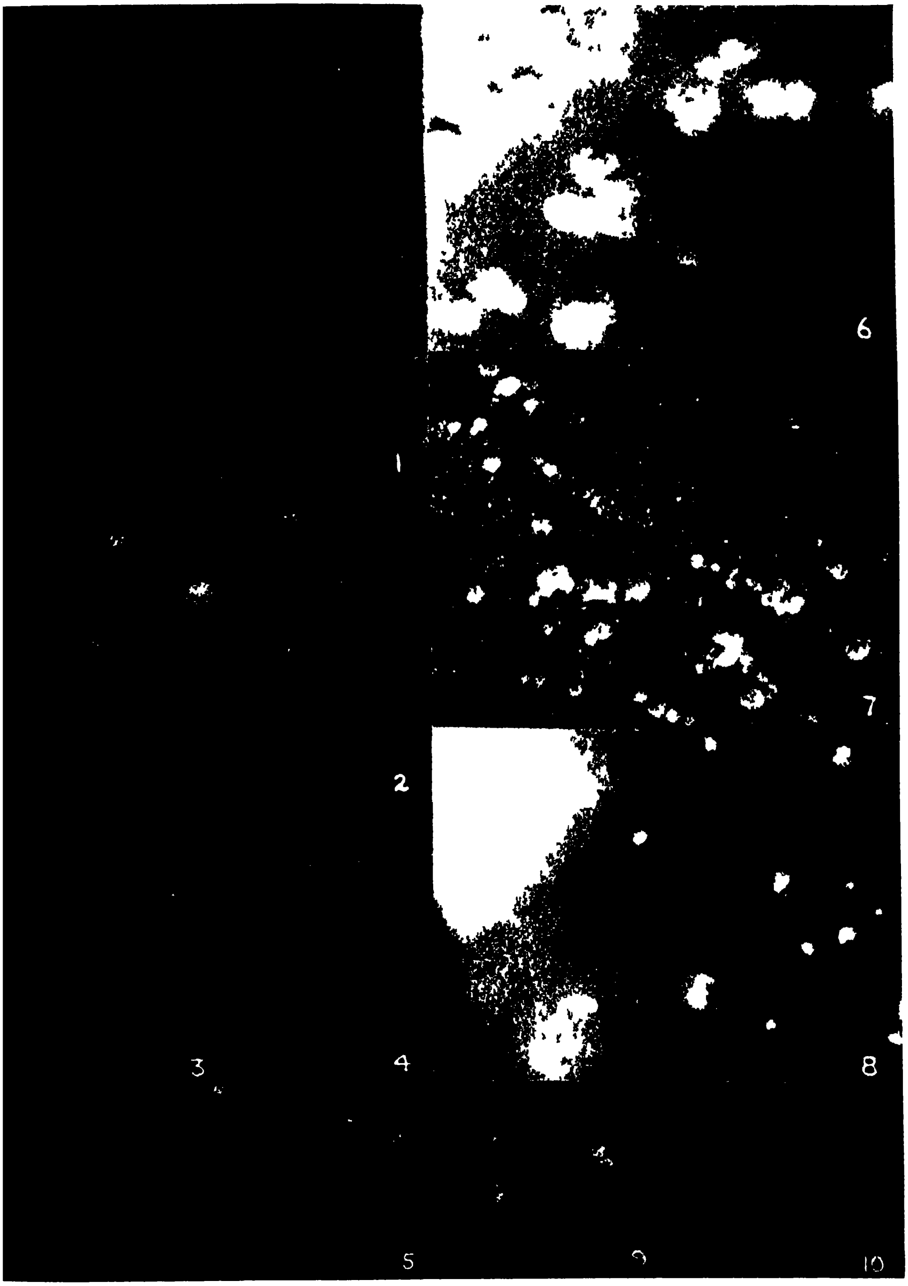
Human—Koch Strain

This culture was obtained from the National Type Culture Collection, London, in 1926. According to the Collection List it is Koch's original strain, although we have not obtained any further history of the culture. During the last five years it has been maintained on glycerol-egg media.

Plated on gentian violet-egg media irregular, granular and spreading S type colonies were produced, Plate I, 7 and 8, Agglutination occurs at pH 3.2. Progressive disease is produced in guinea pigs. This culture then appears to have maintained S characteristics through many years of laboratory cultivation.

Human—H13

This culture is used by Mrs. F. Maltaner of the New York State Department of Health, Albany, in the preparation of antigens for the routine complement fixation reaction for tuberculosis. It has had a long history of high virulence since its isolation in 1909 or 1910 by Krumwiede, late of the New York City Research Laboratories, from a guinea pig injected with tubercular sputum.



Photographs of colonies of tubercle bacilli grown on gentian violet egg media FIG. 1 6 *Colonies from the first culture from six samples of tubercular sputum, four to six weeks growth* FIG. 7 AND 8 S *type colonies, Koch, human strain* FIG. 9 AND 10 S *type, No 13, human*

When received in July 1930 and plated on gentian violet-egg media it produced granular and irregular S colonies (Plate I, 9 and 10). On Proskauer and Beck's fluid it grew as a thin continuous veil which gradually thickened with maturity. Suspensions were agglutinated at pH 3.2 to 3.4. As indicated also by the history of its behavior in guinea pigs it appears to have maintained its S form for some twenty years in laboratory cultures.

Since it came into this laboratory it has been grown for several generations on Sauton's fluid adjusted to an initial reaction of pH 6.2 to 6.4. Successive cultures have shown less of the spreading continuous veil-like pellicle and more heavily wrinkled areas tending to separate into floating islands (Plate III, 33). Gentian violet-egg media plates now show few of the S type of colonies, just noted, but a majority of perfectly smooth, waxy, regular, mound shaped, or R, colonies. These in suspension are agglutinated at pH 3.8 to 4.0 and fail to produce progressive disease in guinea pigs in doses up to 5 mg. This evidently represents an S to R dissociation during less than a year's cultivation on acid Sauton's fluid.

Human—Ceylon

A culture labelled in this manner was obtained from the National Type Culture Collection, London, in 1931 and was said to have been isolated in Ceylon three years ago. Plated on gentian violet-egg media, colonies developed which for the most part were definitely R in type (Plate II, 11) although a very few showed a granular spreading margin. Suspensions are agglutinated at pH 3.8 to 4.0. Guinea pigs injected with doses up to 5 mg. failed to develop progressive disease. Whatever the original history of this culture may have been it is clearly now avirulent and primarily R in type. The spreading granular margins which have grown out from occasional colonies suggest that an S form might readily be separated.

Human—Nathan Raw-Koch

This presumably is one of Koch's original strains which was sent to Dr. Nathan Raw in 1904 and was received from the National Type Culture Collection, London, in 1930. The culture obtained from London was plated directly on gentian violet-egg media. The colonies were all perfectly smooth waxy mounds without any indication of granular forms or spreading colony margins. This colony form strangely suggests an avian type (Plate II, 13). On liquid media a tendency to diffuse growth also suggests an avian form. Agglutination or emulsions occurred at pH 3.8. Guinea pigs were not infected by doses up to 5 mg.

After this examination was made there came to the attention of the authors a paper by Wilson (24), dealing apparently with the same culture, in which he concludes that this is an avian strain masquerading under the wrong name.

Human—54

According to the history of this culture it was brought to the United States in 1888 from Koch's laboratory by Vaughan. In its 108th transfer it exhibited less than its original virulence for guinea pigs. We obtained it from the New

York State Department of Health, Albany, in 1930. In its present form the culture grows at an entirely atypical rate and degree of luxuriance, reaching a maximum growth on favorable solid or liquid media in four to seven days. When plated on gentian violet-egg media the characteristic R colonies which develop (Plate II, 12) closely resemble those from a rapid-growing bovine strain, described in detail in the first paper of this series (20). In contrast to the several S-type suspensions which, as just indicated, are agglutinated at pH 3.0 to 3.2, suspensions of this R culture were agglutinated at pH 4.2. In guinea pigs 5-mg. doses produced only very slight local lesions which were resorbed in two to three weeks.

After the culture had been grown for a number of generations in large flasks of Proskauer and Beck's synthetic fluid medium, well buffered to pH 7.8 to 8.0, a few thin granular patches of pe'licle appeared in contrast to the typical massive waxy folded growth. On fishing these granular pellicle fragments and transferring to gentian violet-egg plates, colonies appeared which were quite definitely S in form (Plate II, 12A). Suspensions were agglutinated at pH 3.4 as S. These new S forms were highly unstable and very readily reverted to the R form.

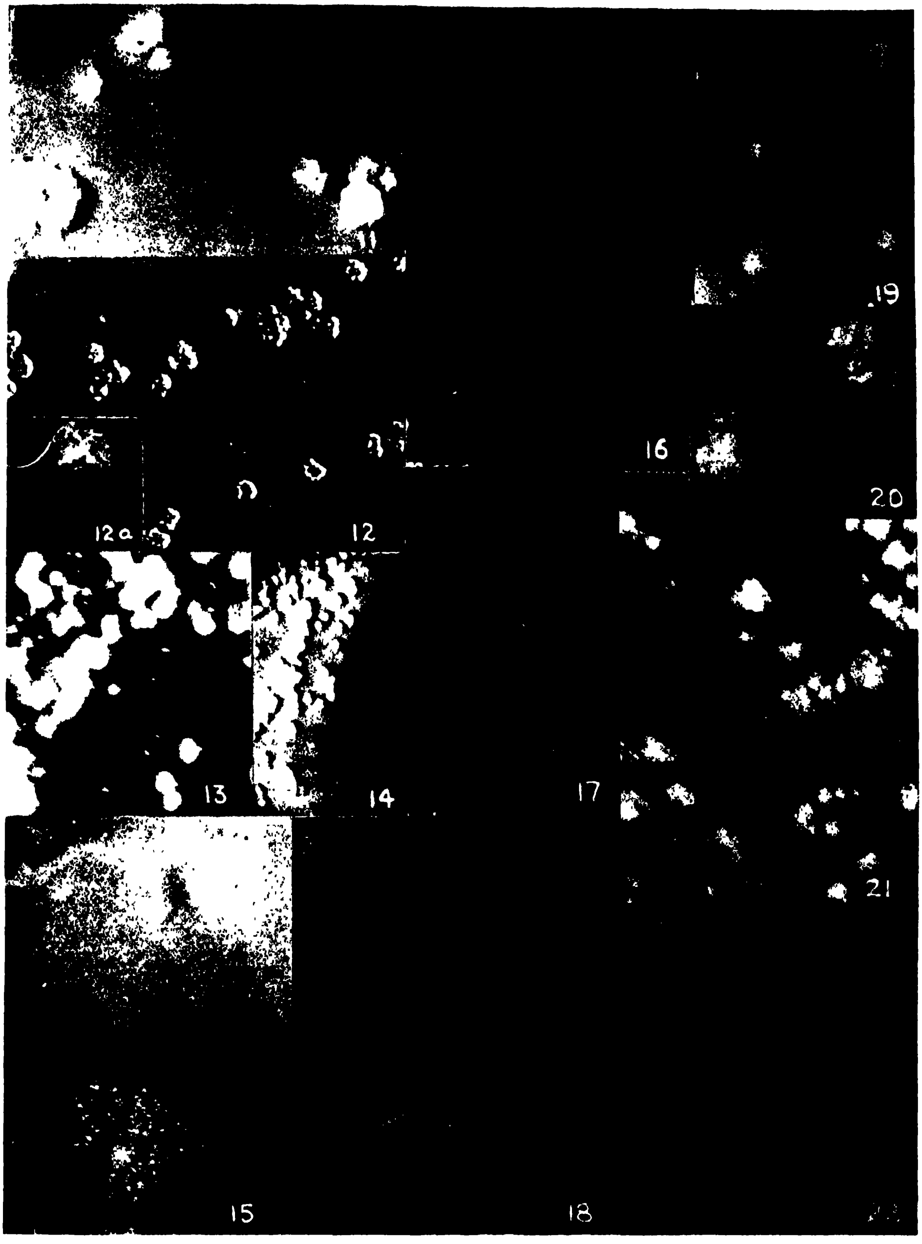
Human—Upt

This culture was isolated by one of us in 1922 from the lung of a girl who died of miliary tuberculosis. No examination of the colonies was made at that time but the culture was used for the next two or three years in class work and for the experimental production of tuberculosis in guinea pigs. After six years of cultivation on glycerol-egg media it was plated on gentian violet-egg. The colonies which appeared and have continued to appear are perfectly regular smooth waxy hemispherical mounds and suggest, as in the case of the Nathan Raw-Koch strain, an avian form (Plate II, 14). Suspensions are agglutinated at pH 4.0 to 4.4. Doses of 5 mg. produce in guinea pigs either no infection or only slight local lesions. It is probable that this is not the culture originally isolated.

Bovine Types

Bovine—Vallée

A culture of the familiar Vallée strain was sent to the authors by Dr. Watson of the Canadian Department of Agriculture, and was carried in our laboratory for about two years on glycerol-potato before the present examination was made. When first plated on gentian violet-egg media the majority of colonies consisted of granular spreading structures with a slightly raised centre and a very thin irregular margin (Plate II, 15 and 16). These colonies closely resembled the S form previously described (20) and were in every way similar to the bovine S described by Petroff (17). The S types in subcultures on gentian violet-egg media have persisted without apparent change for several generations. In Proskauer and Beck's buffered alkaline fluid they grow as a continuous thin pellicle which gradually becomes thickened and folded as the culture matures. Suspensions were agglutinated at pH 2.8. Progressive tuberculosis was produced in guinea pigs. This apparently represents the characteristic S, virulent, bovine form.



Photographs of colonies of tubercle bacilli grown on gentian violet-egg media. FIG. 11. *R type, Ceylon, human.* FIG. 12. *R type, No. 54, human.* FIG. 13 AND 14. *Avian-like colonies with a questionable history of human strains (see text).* FIG. 15 AND 16. *S type, Vallee bovine strain.* FIG. 17 AND 18. *R type, Vallee bovine.* FIG. 19 AND 20. *S type, No. 56, bovine.* FIG. 21. *R type, Calmette, N. Raw, bovine.* FIG. 22. *R type, Brown, bovine.*

The original plates made of the Vallée strain, after the authors had carried the culture for two years on potato media, in addition to the S forms just mentioned exhibited a very small proportion of R colony types, 1 R to 5000 or 10,000 S. These R colony forms grew initially as regular hemispherical mounds with a slightly pebbled surface, regular outline and rising abruptly from the surface of the medium (Plate II, 17 and 18). Later they became umbilicated, or folded, similar to those described in detail for the R bovine 599 (20). In Proskauer and Beck's fluid, growth occurred in island-like masses rather than in the continuous veil form of the S. Suspensions were agglutinated at pH 3.8. Doses up to 5 mg. produced conspicuous local lesions but failed to produce a progressive disease in guinea pigs.

It seems apparent that this bovine strain which has long maintained a high degree of virulence underwent a partial S to R dissociation with accompanying loss of virulence previous to or during our two years of cultivation on potato media.

Bovine—561

This culture is probably one of those described by Ravenal (18), and has a long history of high virulence. We received it from the New York State Department of Health in 1930. Plates grown from the original culture received exhibited only the typical granular spreading S colonies, (Plate II, 19 and 20), similar to those of the S form of the Vallée strain. Cultures on Proskauer and Beck's fluid develop as a thin continuous veil gradually thickening into a folded pellicle. Small doses in guinea pigs produced progressive tuberculosis and death in three to five weeks. Suspensions were agglutinated at pH 2.8.

Bovine—Calmette-Nathan Raw

A culture of unknown history with this designation was received from the National Type Culture Collection in 1930. The original plates exhibited for the most part smooth hemispheres or waxy folded R colonies with very few showing granular spreading margins (Plate II, 21). Suspensions were agglutinated at pH 4.2. Doses up to 5 mg. failed to produce progressive disease in guinea pigs. This is evidently a culture principally R but the spreading colony margins, although few, suggest that an S form might be readily developed.

Bovine—Brown

Dr. Caulfield and Dr. Brown of the Connaught Laboratories, Toronto, very kindly supplied the authors with a culture of bovine origin. This culture, they state, exhibited a fairly high degree of virulence when freshly isolated but after several years of laboratory cultivation the virulence has been so far lost that it produces no demonstrable lesions in guinea pigs though the guinea pigs become sensitive to tuberculin. The first culture received from Dr. Brown, plated on gentian violet-egg media, produced characteristic R colonies rising abruptly from regular outlines at the surface of the medium to form slightly pebbled hemispherical colonies indistinguishable from the previously described R Vallée (Plate II, 22). Agglutination of emulsions occurred at pH 4.0.

Bovine—B.C.G.

Several cultures of B.C.G. have been under observation for a number of

years. The typical culture as noted by Petroff and Steenken (17), Begbie (1), Kraus (7), Lange (8), Gerlach (4), Tzeknovitzer (21) and others when plated on gentian violet-egg media produces colonies with a smooth waxy or slightly pebbled surface and regular outline on the surface of the medium though fully mature colonies frequently become considerably folded—the typical R colony form (Plate III, 23 and 24). Growths of this form have been repeatedly obtained. The definite R colonies when injected into guinea pigs even in large doses have produced only localized lesions. Suspensions are agglutinated at pH 4.0, a pH level, as the previous results indicate, characteristic of R forms. Detailed data concerning S forms which have been separated from several B.C.G. cultures will be presented in a separate paper.

Bovine—599

In the first paper of this series (20), it was shown that a rapid-growing bovine strain consisted entirely of R colony forms, but that continued growth on alkaline fluid media resulted in the separation of somewhat atypical S forms.

Avian Cultures

Petroff's Avian R and S

Petroff (17) reported that a strain of avian organisms which he had had under cultivation for a number of years had been dissociated into R and S types. The S he found highly virulent for chickens and the R avirulent. Through the kindness of Dr. Petroff the authors received these dissociated R and S forms more than a year ago. Cultivation on a gentian violet-egg medium has resulted in the formation of colonies similar to those described and figured by Petroff (Plate III, 24 and 25). In 5-mg. doses the S produced progressive disease in pigeons; similar doses of the R failed to produce demonstrable infections.

Kahn and Schwarzkopf (6) working with Petroff's cultures have just shown that the R and S also differ in electrophoretic potentials. We found that R was agglutinated in buffer solutions at pH 3.6 and the S at pH 2.8 which is in approximate agreement with Kahn and Schwarzkopf's electrophoretic potential results.

Six avian strains of unknown history were recently obtained; four in 1928 (823, 807, 116, 118) from the American Type Culture Collection, and two from the Lister Institute Collection, No. 120 in 1926 and Bang in 1930. These were all cultivated for several generations on glycerol-egg before this study was undertaken.

Avian 823 and 807

These two cultures plated on gentian violet-egg media produced perfectly regular, smooth, mound-like colonies (Plate III, 27 and 28), indistinguishable in appearance from the S type of the Petroff culture. In Proskauer and Beck's fluid media the growth was diffuse with very little tendency to produce a pellicle. Suspensions of No. 823 were agglutinated at pH 2.8 and in pigeons 5-mg. doses produced progressive disease. Suspensions of the 807 agglutinated at a much higher pH, 3.4, and though progressive disease was produced in pigeons the development was much slower.

Fig. 23-32. *Photographs of colonies of tubercle bacilli grown on gentian violet-egg media.* Fig. 23 and 24. *R type, B.C.G., bovine.* Fig. 25. *S type, Petroff's avian strain.* Fig. 26. *R type, Petroff's avian.* Fig. 27 and 28. *S type, No. 823 and 807, avian.* Fig. 29 and 30. *R type, No. 116 and 118, avian.* Fig. 31 and 32. *Intermediate colony types, No. 120 and Bang, avian.* Fig. 33. *Photographs of cultures of human strain No. 13 growing on Proskauer and Beck's fluid; on the left, veil-like S growth with R-like islands; on the right, the thicker R growth.* Fig. 34. *B.C.G.; on the left, the typical R growth; on the right, the veil-like S growth.*

Avian 118 and 116

Gentian violet-egg plates of these two cultures exhibited typical R colonies (Plate III, 29 and 30) indistinguishable from the R forms of Petroff's culture and strikingly unlike Petroff's S forms and those of No. 823 and 807 just described. Both of these in fluid media grew as a surface pellicle in contrast to the diffuse habit of the S and both were agglutinated at a high pH, 3.8 to 4.0. In 5-mg. doses both failed to infect pigeons.

Avian—Bang and 120

The colonies developing from these strains were much less definite than the previously described avian cultures. A few exhibited S characteristics, some were R-like but the majority appeared to be of an indefinite intermediate form (Plate III, 31 and 32). In fluid media growth resembled the R, that is a definite pellicle formed in contrast to the diffuse growth of the S, while suspensions like the R types agglutinated at a high pH, 4.0. In pigeons 5-mg. doses failed to produce demonstrable infection.

Discussion

The data presented in the foregoing sections have been summarized in Table I. It seems evident, as shown in Petroff's work, that there is a definite correlation between colony structure and virulence of human, bovine and avian tubercle bacilli. It has been shown that freshly isolated virulent strains exhibit the S colony structure and at the same time all the cultures examined which have had a long history of high virulence grow on solid media in a similar form. It therefore seems evident that the two characteristics have been maintained in parallel. In contrast all the cultures which exhibit lack of virulence or have a history of loss of virulence grow in R colonies. The instances quoted in this paper, in previous papers in this series and in Petroff's work on change from S to R with accompanying decrease or loss of virulence under experimentally controlled conditions, probably represent the sequence of events in the several cultures with a history of loss of virulence reported on in the previous sections.

Although it has not been studied in the same detail it appears that all the virulent or S human and bovine forms grow in fluid media in the form of a continuous thin and spreading veil which gradually thickens into a heavy folded pellicle, whereas the avirulent or R forms grow as thickened island-like masses. The virulent or S avian forms all appear to grow in fluid media in a diffuse manner in contrast to the avirulent or R forms which grow as a surface pellicle.

The results with acid agglutination show an equally definite correlation between virulence and colony structure. Of the human strains, ten S types were agglutinated at approximately pH 3.2 and five R types at pH 3.8 to 4.2; two bovine S types at pH 3.2 and five bovine R types at pH 4.0 to 4.2; four avian S forms at pH 2.8 to 3.4 and four R forms at pH 3.6 to 4.0. Freud reported that certain stock cultures of tubercle bacilli which had retained virulence for guinea pigs were agglutinated at pH 2.8 to 3.0. Acid agglutina-

tion provides a measure of potential difference (Northrop and DeKruif; Northrop, 11) though probably less definite than direct determination of electrophoretic migration or isoelectric points. The agreement of the acid agglutination results on Petroff's avian R and S forms with Kahn and Schwarzkopf's potential determinations on the same forms and our own potential studies on these and other cultures appear to confirm the theoretical relationship of acid agglutination and potential.

These results appear to be in conformity with the recent demonstration of a relationship between potential difference and virulence in the case of *Pneumococci*, diphtheria bacilli, and other species (5). The early work on acid agglutination, Michealis (10) Beniasch (2), and the more recent work on potential by Falk (5), clearly indicate that potential measurements give no direct indication of virulence, but that within the confines of a species which contains pathogenic and non-pathogenic forms or forms of high and low virulence, the pathogenic or most virulent types exhibit higher potentials than the non-pathogenic or forms of lower virulence. In this instance it seems evident that comparison of acid agglutination of avian and human or avian and bovine tubercle bacilli gives no significant indication of the relative virulence. But it does appear that comparison of the acid agglutination points of a group of cultures of avian or a group of cultures of bovine bacilli or human bacilli does provide an indication of the degree of relative virulence within the group as precise as does a comparison of the colony structure.

The tests of virulence mentioned throughout this paper provide evidence of differences between those cultures which produce definite and rapidly progressive tuberculosis in experimental animals demonstrable in a few weeks, in contrast to those cultures which in rather large doses fail to produce demonstrable progressive disease in this relatively short period. The determinations do not take into consideration the possibility of progressive disease developing after long periods of incubation, as has been so clearly demonstrated to occur in certain instances by Watson (23), nor do they allow for the possibility of progressive disease developing in occasional animals in large groups as described by Uhlenhuth and Seiffert (22) and considered to result from exceptionally low natural immunity. This examination of virulence, therefore, is in no sense germane to the important problem of alteration in virulence of organisms in the body of infected organisms.

This examination, including twenty-eight cultures of tubercle bacilli from widely differing sources, provides evidence that the phenomenon of dissociation and the differentiation of two or more types of the tubercle bacilli, as previously described, is probably of general application and not only of application to special cases, such as B.C.G. cultures. Detailed study of several of the types mentioned is now in progress and will be reported on in the near future.

References

1. BEGBIE, R. S. Edinburgh Med. J. 37: 187-191. 1930.
2. BENIASCH, M. Z. Immun. Forsch. 12: 268-297. 1912.
3. DEKRUIF, P. H. J. Gen. Physiol. 4: 639-654. 1922.

4. GERLACH, F. Ergebn. n. d. Hyg. Bakt. Immunit. u. exper. Therap. 824. 1930.
5. JORDON, E. O. and FALK, I. S. Newer knowledge of bacteriology and immunology. Univ. Chicago press, 1928.
6. KAHN, M. C. and SCHWARZKOPF, H. Am. Rev. Tuberculosis, 23: 45-55. 1931.
7. KRAUS, R. Z. Immunitäts. 61: 454-459. 1929.
8. LANGE, B. Deut. Med. Wochschr. 56: 927-929. 1930.
9. MCCUTCHEON, M., MUDD, S., STRUMIE, M. and LUCKÉ, B. J. Gen. Physiol. 13: 669-681. 1930.
10. MICHEALIS, L. Deut. med. Wochschr. 37: 969-972. 1911.
11. NORTHROP, J. H. in JORDON, E. O. and FALK, I. S. Newer knowledge of bacteriology and immunology. Univ. of Chicago Press, 1928.
12. PETROFF, S. A. Johns Hopkins Hosp. Bull. 26: 276-287. 1915.
13. PETROFF, S. A. Proc. Soc. Epxtl. Biol. Med. 24: 632-634. 1927.
14. PETROFF, S. A. Proc. Soc. Exptl. Biol. Med. 24: 956-958. 1927.
15. PETROFF, S. A., BRANCH, A. and STEENKEN, W. Proc. Soc. Exptl. Biol. Med. 25: 14-17. 1927.
16. PETROFF, S. A., BRANCH, A. and STEENKEN, W. Am. Rev. Tuberculosis, 19: 9-46. 1929.
17. PETROFF, S. A. and STEENKEN, W. J. Exptl. Med. 51: 831-845. 1930.
18. RAVENAL. Univ. Penn. Bull. 1901-1902.
19. REED, G. B. J. Bact. (In Press).
20. REED, G. B. and RICE, C. E. Can. J. Research, 4: 389-398. 1931.
21. TZEKNOVITZER, M. Ann. inst. Pasteur, 42: 67-75. 1928.
22. UHLENHUTH, P. and SEIFFERT, W. Z. Immunitäts. 69: 186-212. 1930.
23. WATSON, E. A. J. Am. Vet. Med. Assocn. 73: 799-816. 1928.
24. WILSON, G. S. J. Path. Bact. 28: 69-96. 1931.

TABLE I

RESULTS OF X-RAY TREATMENT OF R TYPES

Expt. No.	Source of organisms	Colony forms growing from suspension of organisms		Expt. No.	Source of organisms	Colony forms growing from suspension of organisms	
		Before X-raying	After X-raying			Before X-raying	After X-raying
1	1 R colony	All R	Mostly R, very few S	4	1 R colony	All R	All R
	1 R colony	All R	40% R, 30% S, 30% intermediate		1 R colony	All R	All R
	1 R colony	All R	All R		1 R colony	All R	Mostly R, very few S, very few intermediate
2	1 R colony	All R	Mostly R, very few S, very few intermediate		1 R colony	All R	Mostly R, very few S
	1 R colony	All R	All R	5	1 R colony	All R	All R
3	1 R colony	All R	Mostly R, very few intermediate		1 R colony	All R	98% R, 2% S
	1 R colony	All R	Mostly R, very few intermediate		1 R colony	99% R 1% intermediate	95% R, 5% S
	1 R colony	All R	Mostly R, very few intermediate		1 R colony	All R	All R
	1 R colony	All R	Mostly R, very vew intermediate		1 R colony	All R	All R
	1 R colony	All R	All R		1 R colony	98% R 2% intermediate	90% R, 2% S, 8% intermediate

NOTE:—*Single colonies were emulsified in saline, filtered through paper and plates made before and after X-raying.*

dissociation, while after irradiation 12, or 60%, showed similar or in most cases much more definite dissociation.

The S and intermediate colonies which appeared on the plates made from radiated suspensions exhibited much the same type of variability as similar S colonies which grew from alkaline broth cultures, as discussed in the previous paper concerning this organism (20). An S colony arising from a series consistently R for a considerable previous period generally produced, when suspended in fluid and plated on gentian violet-egg, from 10 to 90% S and the balance intermediate or R types. Repeated fishing and replating of S forms generally increased the percentage although as previously shown the S forms were highly unstable on this medium. By exposing suspensions of the selected

TABLE II

EFFECT OF TREATING TWO-WEEK-OLD CULTURES OF BOVINE 599 PREVIOUSLY TREATED WITH X-RAYS TO A SECOND IRRADIATION FOR 12 MIN.

Culture	Subculture					Type of colony
	1	2	3	4	5	
Control	37	33	50	40	50	% R
	50	19	10	20	5	% S
	13	50	40	40	45	% RS
X-rayed	5	3	7	10	0	% R
	20	45	93	90	100	% S
	75	52				% RS

colony to X-rays, before plating, as in the previous experiments, the isolation of S types was facilitated. In a representative instance an apparently S colony was suspended and a sample plated; from this plate a second apparently S colony was selected, and so on through five culture generations with the indifferent results indicated in the control, Table II. A similar colony apparently S was suspended but irradiated before plating, and from this plate a second apparently S was suspended in fluid and X-rayed and this repeated through five culture generations. The results shown in Table II indicate a much higher yield of S than in the non-radiated control. Comparable results were obtained in several instances.

III. Differences in Stability of R Colonies

In the previous paper concerning this organism, it was shown that when R colonies, apparently similar, were selected and exposed to the same cultural conditions they did not exhibit the same degree of variability; some continued to produce only R colonies, others produced S colonies and intermediate colonies at varying rates. The same was shown in Section II to be true of X-rayed suspensions. Gates (5) found that apparently similar bacteria varied in respect to their sensitivity to ultra-violet light, and similar observations have been made in respect to many agencies. This might be explained on the

TABLE III

EFFECT OF TIME OF EXPOSURE TO X-RAYS UPON CULTURES OF BOVINE 599

Culture	Time, min.	Strength of current, milliamperes	Dosage, m. amp. min.	Organisms per cc. of emulsion	Per cent killed
Control	—	—	—	1,696,000	—
X-rayed	5	3	15	840,000	50
X-rayed	10	3	30	525,000	69
X-rayed	15	3	45	382,000	78
X-rayed	20	3	60	296,000	83

assumption of different degrees of fixity in the individual organisms of those characteristics which contribute to the visible form of the colony. The difference might result from the fact that the colony capable of producing variability contains two or more types of individual organisms, and as in plating one or more of the types might not be included, their characteristic colony type would therefore not appear on the plates. The latter suggestion would appear to be inadequate to explain the fact noted in the previous paper that over a period of two years this organism exhibited conspicuous variability. Two years later the R strains, which had been carried during this period by transferring every one to two months on slants of gentian violet-egg media, appeared to have become perfectly stable and exhibited no dissociation in fluid media in which they had previously dissociated. At this period the X-ray treatment which had earlier promoted variability, failed to produce any change in the stability of the R forms.

IV. The Influence of Dosage of X-rays

Several experiments have indicated that the dosage used is one of considerable toxicity. Suspensions of R forms were exposed to various intensities of X-rays and plate counts made of the surviving bacteria. Table III indicates the results of varying the time of exposure and Table IV of varying the time and the amperage. In both cases the voltage remained at approximately

TABLE IV

THE EFFECT OF THE INTENSITY OF IRRADIATION UPON CULTURES OF BOVINE 599

Culture	Strength of current, m. amp.	Fall in potential, volts	Time, min.	Dosage, m. amp. min.	Number of organisms per cc.	Per cent killed
Control	—	—	—	—	428,000	—
X-rayed	2	88,000	10	20	129,000	70
X-rayed	4	82,000	5	20	137,000	68
X-rayed	3	84,000	10	30	108,000	75
X-rayed	4	82,000	10	40	43,600	90
X-rayed	5	79,000	10	50	14,000	97

88000. It is apparent from the tables that the lethal effect is considerable and that it is proportional to the dosage. Applying these dosages to R strains, which exhibited no dissociation in successive cultures in alkaline broth,

resulted in no change in the form of the colonies developing from the surviving organisms.

V. The Effect of Protective Colloids

McKinley and Fisher (18) found that the presence of normal rabbit serum protected bacteriophage and viruses from the lethal effects of ultra-violet light. Meyer (15) found a similar lack of bactericidal action in the presence of haemoglobin. Harris and Hoyt (8) investigated the absorption capacity of a number of substances and found that aromatic radicals possessed this property to a marked degree. Eidinow (4), in studying the bactericidal action of light, found that bacilli mixed with blood and exposed in a very thin film or in a mixture of defibrinated rabbit blood were not killed in one hour.

In order to determine the protective effect, if any, of serum and gelatine, these substances were added to a suspension of the *Mycob. tuberculosis bovis* No. 599 organisms, known to consist of both R and S types. They were then exposed to the dosage of X-rays used in the previous experiments. The results are shown in Table V. In the radiated preparation without protein, as in the former cases quoted, the proportion of S types was increased over the unexposed control. Where either 1% gelatine or 1-10 blood serum was added,

TABLE V

THE EFFECT OF PROTECTIVE COLLOIDS UPON THE SENSITIVITY OF BOVINE 599 TO X-RAY TREATMENT

Culture	Protein	R colonies %	S colonies %	Intermediate colonies %
Control	0	34	66	0
X-rayed	0	10	86	4
Control	Gelatine	21	75	4
X-rayed	Gelatine	26	58	16
Control	Blood serum, 1-10	41	59	0
X-rayed	Blood serum, 1-10	24	66	10
X-rayed	Blood serum, 1-10	35	50	15

however, there was comparatively little difference between the unexposed control and the irradiated preparations. The protein apparently protected the bacteria against the action of the X-rays.

The Effect of X-rays upon Other Species of Bacteria

Meyer (15) reported that tubercle bacilli were not more sensitive to ultra-violet light than other species of bacteria. But as others have pointed out it is probably inaccurate to draw analogies between the effects of different rays, though Meyer (15) demonstrated that cultures resistant to ultra-violet were also resistant to X-rays. Browning and Russ (1) and Gates (5) have shown a definite relationship between the bactericidal effect of light and the absorption spectrum of the bacterial emulsion; Meyer (15) suggests that this may be due to differences in amino complexes in the different organisms.

Several strains of recently isolated *Es. coli* and *Staph. aureus* were exposed to the same dosage of X-rays as used in the previous experiments. No reaction to this X-ray dosage was observed.

A culture of paratyphoid bacilli which had been under observation for a considerable time and which was known to include both R and S colony types was suspended in saline and exposed to the dosage of X-rays used in the previous experiments with the tubercle bacilli and to double this dosage. Plates made from the suspensions before and after irradiation exhibited the same proportion of R and S forms. It seems apparent therefore that an X-ray dosage which does affect certain acid-fast bacteria is without influence upon these representative non-acid-fast bacteria.

Discussion

Several theories as to the action of light upon living matter have been brought forward. Clark (2) developed a photochemical theory postulating that electrons are given off by cell proteins under the influence of ultra-violet light and that these attach themselves to other atoms and molecules with resulting changes in the physical and chemical properties of all the substances concerned. Mayer (14) outlined a very similar explanation; the photochemical rays are absorbed by the reacting substance with a resultant increase in electron activity and liberation of electrons. Since most living particles are negatively charged, this would bring about a gain in positive electricity. Gutfeld and Pincussen (7) also attribute the photochemical effects of light to a loss in negative dispersion and definite physiological disturbance. Hill (10) and Lacassagne and Holweck (12) make somewhat similar suggestions. The inactivation of trypsin by irradiation according to Clark and Northrop (3) seems also to depend upon electrical neutralization. The early work of Young (25) on the reduction of potential in bacterial suspensions after irradiation agrees with these theories.

These observations are very suggestive in view of the finding of several observers that variant types of bacteria exhibit differences in electrical charge. The work just reported by Kahn and Schwarzkopf (11) in which it is shown that S types of avian and bovine tubercle bacilli possess a higher negative charge than the R types is especially interesting in this connection. Work along this line is now in progress and will form the subject of a further paper in this series in the near future.

Acknowledgments

The authors are indebted to Dr. Jones and Dr. Richards, radiologists at the Kingston General and the Toronto General Hospitals, respectively, for the use of X-ray equipment, many acceptable suggestions and other courtesies.

References

1. Browning, C. H. and Russ, S. Proc. Roy. Soc. Lond. Ser. B. 90: 33-38. 1919.
2. Clark, G. L. Colloid symposium monograph (Weiser). v. 4. 1926.
3. Clark, H. and Northrop, J. H. J. Gen. Physiol. 9: 87-96. 1925.

4. EIDINOW, A. Brit. Med. J. 2: 160-161. 1927.
5. GATES, F. L. Proc. Soc. Exptl. Biol. Med. 21: 761-762. 1923.
6. GOODSPEED, T. H. and OLSON, A. R. Proc. Natl. Acad. Sci. 14: 66-69. 1928.
7. GUTFELD, F. and PINCUSSEN, L. Centr. Bakt. Parasitenk. Orig. Abt. 1. 109: 107-112. 1928.
8. HARRIS, F. I. and HOYT, H. S. Univ. California Pub. Pathology, 2:245-250. 1919.
9. HENRI, V. Compt. rend. soc. biol. 73: 321-322. 1912.
10. HILL, L. Proc. Roy. Soc. Med. 17: 15-21 (Sec. on Electrotherapeutics). 1924.
11. KAHN, M. C. and SCHWARZKOPF, H. Am. Rev. Tuberculosis, 23: 45-55. 1931.
12. LACASSAGNE, A. and HOLWECK. J. Am. Med. Assocn. (Note) 90: 2046-2047. 1928.
13. LACASSAGNE, A. and PAULIN, A. Compt. rend. soc. biol. 93: 1502-1503. 1925.
14. MAYER, E. Clinical application of sunlight and artificial radiation, including their physiological and experimental aspects, with special reference to tuberculosis. Williams and Wilkins, 1926.
15. MEYER, P. S. Klin. Wochschr. 2: 297-299. 1923.
16. MULLER, H. J. Am. Naturalist, 60: 192-195. 1926.
17. MULLER, H. J. Science, 66: 84-87. 1927.
18. MCKINLEY, E. B., FISHER, R. and HOLDEN, M. Proc. Soc. Exptl. Biol. Med. 23: 408-412. 1926.
19. NADSON, G. and PHILIPPOV, G. Compt. rend. soc. biol. 98: 366-368. 1928.
20. REED, G. B. and RICE, C. E. Can. J. Research, 4: 389-398. 1931.
21. STADLER, L. J. Proc. Natl. Acad. Sci. 14: 69-75. 1928.
22. SCHNEIDER, E. C. Klin. Wochschr. 5: 1708-1710. 1926.
23. SCHEPMANN, W. and FLECKE, H. Klin. Wochschr. 5: 1608-1611. 1926.
24. STRANGEWAYS, T. S. P. and HOPWOOD, F. L. Proc. Roy. Soc. Lond. Ser. B. 100: 283-293. 1926.
25. YOUNG, S. W. Proc. Soc. Exptl. Biol. Med. 10: 151-153. 1913.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL *of* CANADA

VOLUME 5 | AUGUST, 1931 | NUMBER 2

LONGITUDINAL AND RADIAL VIBRATIONS IN LIQUIDS CONTAINED IN CYLINDRICAL TUBES[1]

BY GEORGE S. FIELD[2]

Abstract

I. The boundary conditions at the tube wall are considered, taking into account the natural frequency of the wall itself. An expression is derived for the radial displacement of the wall due to the excess pressure acting in the liquid. II. A formula is developed for the resonant frequencies of radial vibration in the cylinder of liquid. For tubes of ordinary size these lie well up in the ultrasonic frequencies. III. The character of a longitudinal sound wave in tubes of liquid is analyzed. Selective absorption of the longitudinal vibration is found to occur at the natural frequency of the radial vibration of the column of liquid. Examination of the expression for particle velocities reveals that the vibration for low frequencies is of a different type from that for high.

Introduction

The following theoretical investigation is an attempt to explain certain experimental results which were observed by Boyle and Froman (1) while they were studying the propagation of sound through liquids contained in cylindrical tubes.

Although existing theory has explained fairly satisfactorily the diminution in wave velocity almost invariably observed by previous experimenters, an increased velocity such as was noted by Boyle and Froman seems to have been unaccounted for. In the following pages it will be shown that this phenomenon is due to selective absorption, caused by the energy of the longitudinal wave being absorbed by a radial vibration occurring at the natural frequency of this vibration.

In this discussion the case considered is that of an infinite cylinder of liquid. The viscosity of the liquid is neglected, and the walls of the tube are considered to be thin, though the effect of a thicker tube wall is discussed for approximate boundary conditions.

[1] *Manuscript received May 27, 1931.*
Contribution from the National Research Laboratories, Ottawa. Read before Section III of the Royal Society of Canada in May, 1931.

[2] *Junior Research Physicist, National Research Laboratories, Ottawa.*

Part I. Boundary Conditions at Wall of Tube

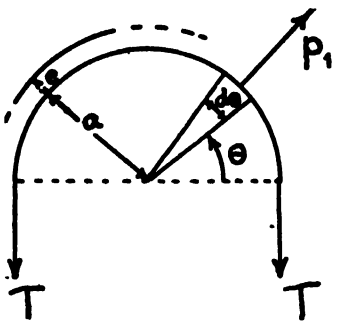


FIG. 1. *Half-section of tube wall showing pressure p_1 balanced by tension T.*

In Fig. 1, suppose the radius (a) of the tube increases by an amount e, on account of the excess pressure (δp) in the liquid at the boundary. Let the pressure necessary to produce the increase in radius be denoted by p_1.

If we consider a section of the tube, one unit long, we see that the pressure on one-half the tube is balanced by the tension (T) in the fibres of the tube wall (see Fig. 1).

Or, we have, $2T = 2\int_0^{\frac{\pi}{2}} a\,d\theta\, p_1 \sin\theta = 2\,ap_1$

Whence $\quad T = ap_1$

Now the increase in circumference due to a tension T is given by, $\epsilon = \dfrac{Tl}{AE}$

Where T = force acting; l = original length $= 2\pi a$; A = cross-sectional area = $h \times 1$, where h = thickness of wall. E = Young's modulus.

In this relation, Young's modulus should be corrected to compensate for the fact that the stresses in the tube wall are rapidly alternating, so that the deformation of the wall does not quite attain the statical value corresponding to the instantaneous distribution of pressure in the liquid. Korteweg (3) suggests the following value be used,

$$E^1 = E\left(1 - \frac{5h}{6a}\right)$$

Our equation then becomes,

$$\epsilon = \frac{Tl}{AE^1}$$

Substitute $T = ap_1$, and $\epsilon = \dfrac{ap_1 \cdot 2\pi a}{h\,E^1} = \dfrac{2\pi a^2 p_1}{hE^1}$

Increase in radius $= e = \dfrac{\epsilon}{2\pi}$

$$= \frac{a^2 p_1}{hE^1}$$

We now have the relation, $e = \dfrac{a^2 p_1}{hE^1}$ or $p_1 = \dfrac{ehE^1}{a^2}$

Of the excess pressure at the boundary (δp), part is used up in producing the extension e, and part is used up in producing a change in momentum of the tube wall $\left(= m\dfrac{d^2 e}{dt^2}\right)$ and also in opposing friction $\left(= r\dfrac{de}{dt}\right)$. In these relations m = mass of wall per unit area, corresponding to δp = force per unit area, and r = a constant of proportionality relating the frictional force opposing the

motion of the wall to the velocity of the latter. We here assume, as is usual, that the frictional force is directly proportional to the velocity.

Let ρ_1 = density of the material of the wall.

Then mass per unit area $(=m) = \rho_1 h$.

We now have the following equation, $\delta p = p_1 + m\dfrac{d^2e}{dt^2} + r\dfrac{de}{dt}$

$$\text{or, } \delta p = \frac{hE^1}{a^2} e + \rho_1 h \frac{d^2e}{dt^2} + r\frac{de}{dt} \tag{1}$$

But $\delta p = \rho\dot{\phi}$ (4)

Where ϕ = velocity potential existing for liquid, and $\dot{\phi} = \dfrac{\partial\phi}{\partial t}$

Hence, from equation (1), $\rho\dot{\phi} = \dfrac{hE^1}{a^2} e + \rho_1 h \dfrac{d^2e}{dt^2} + r\dfrac{de}{dt}$

$$\text{or, } \frac{d^2e}{dt^2} + \frac{r}{\rho_1 h}\frac{de}{dt} + \frac{E^1}{\rho_1 a^2} e = \frac{\rho}{\rho_1 h}\dot{\phi} \tag{2}$$

The general solution of equation (2) is,

$$e = \epsilon^{-\alpha t}\left[C \cos p_0 t + D \sin p_0 t\right] + \frac{\rho\dot{\phi}}{\rho_1 h[\beta^2 - \omega^2 + 2i\alpha\omega]} \tag{3}$$

Where, ϵ = base of Napierian logarithms; C and D are constants; $p_0 = \sqrt{\beta^2 - \alpha^2}$; $\beta^2 = \dfrac{E^1}{\rho_1 a^2}$; $2\alpha = \dfrac{r}{\rho_1 h}$; $\omega = 2\pi \times$ frequency.

Equation (3) represents the way the wall of the tube moves in and out with time. The first part of e is a transient solution, since the damping factor ($\epsilon^{-\alpha t}$) will soon damp out this part of the vibration.

Note that $\dfrac{p_0}{2\pi}$ is the frequency of the resonant vibration of the tube wall, and, $p_0 = \sqrt{\beta^2 - \alpha^2}$: α will be very small compared with β, and hence

$$p_0 \doteq \sqrt{\beta^2} = \beta = \frac{1}{a}\sqrt{\frac{E^1}{\rho_1}} \tag{4}$$

Equation (4) is the same as is given by Lamb*.

The steady state value of e is the one we need for our boundary conditions, *i.e.*,

$$e = \frac{\rho\dot{\phi}}{\rho_1 h[\beta^2 - \omega^2 + 2i\alpha\omega]}$$

But $\beta^2 = p_0^2 + \alpha^2$

Hence
$$e = \frac{\rho\dot{\phi}}{\rho_1 h[p_0^2 - \omega^2 + \alpha^2 + 2i\alpha\omega]} \tag{5}$$

Note that when the period of the pressure variations corresponds to that of the natural frequency of the tube wall, the displacement (e) becomes very large, limited only by the resistance factor ($\alpha^2 + 2i\alpha\omega$).

**Reference (4, p. 138); for the case, s = 0.*

Part II. Resonant Frequencies of Radial Vibration in a Cylinder of Liquid

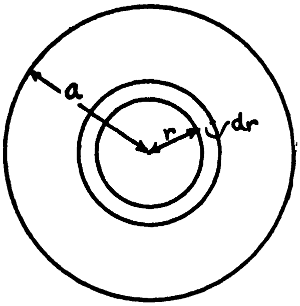


FIG. 2. *Section of cylinder of liquid.*

The method of developing the differential equation is similar to that adopted by Crandall (2, p. 87) in discussing *plane* waves of sound.

Consider a portion of the cylinder of unit length, radius a.

Take an annulus, width dr, at a distance r from the axis.

The flow into the annular ring is given by, $\rho\xi 2\pi r\, dt$

Where ρ = density of the liquid and ξ = particle velocity.

The flow out of the ring is, $2\pi\, dt \left\{ r\rho\xi + \frac{\partial}{\partial r}(r\rho\xi)\, dr \right\}$

Hence net flow in during time $dt = -2\pi\, dt \frac{\partial}{\partial r}(r\rho\xi) dr$

Also increase in fluid contained $= 2\pi r dr\, \frac{\partial \rho}{\partial t} \cdot dt$

And we have finally, $2\pi\, dr \cdot dt\, \frac{\partial}{\partial r}(r\rho\xi) dr + 2\pi r\, dr\, dt \frac{\partial \rho}{\partial t} = 0$

Or
$$r\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial r}(r\rho\xi) = 0 \tag{6}$$

Which is the equation of continuity.

Consider a piece of the annular ring, of unit thickness, width dr one way and $rd\theta$ the other.

The force out due to harmonic pressure (p) is,

$$p \cdot rd\theta + \left[2\, \frac{p + p + \frac{\partial p}{\partial r} \cdot dr}{2} \cdot \sin\frac{d\theta}{2} \right] dr$$
$$= p\, r\, d\theta + p\, dr\, d\theta$$

The force in $= \left(p + \frac{\partial p}{\partial r} \cdot dr\right)\left(r + dr\right) d\theta$

$$= p\, r\, d\theta + p\, dr\, d\theta + r\, \frac{\partial p}{\partial r} \cdot dr \cdot d\theta$$

Net force out $= -r\, \frac{\partial p}{\partial r} \cdot dr \cdot d\theta$

Rate of change of momentum $= \rho\, rd\theta\, dr\, \frac{\partial \xi}{\partial t}$

Hence we have, $r\frac{\partial p}{\partial r} + \rho r \frac{\partial \xi}{\partial t} = 0$

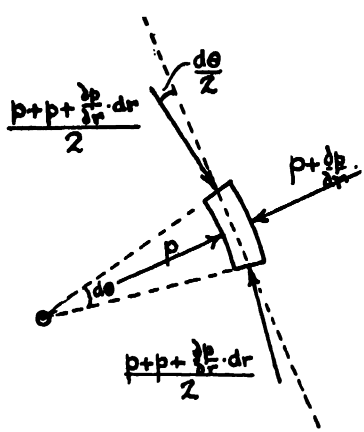


FIG. 3. *Element of liquid showing pressures acting.*

$$\text{or,}\quad \frac{\partial p}{\partial r} + \rho\, \frac{\partial \xi}{\partial t} = 0 \tag{7}$$

Which is the equation of motion.

In Equation (6) substitute $\rho=\rho_0(1+s)$, where s = condensation.
In Equation (7) substitute $p=Ks$, where K = coefficient of cubic elasticity.
Neglect $s\xi$ compared with ξ (2, p. 87).

Then (6) leads to, $$r\frac{\partial s}{\partial t}+r\frac{\partial \xi}{\partial r}+\xi=0 \tag{8}$$

(7) leads to, $$K\frac{\partial s}{\partial r}+\rho\frac{\partial \xi}{\partial t}=0 \tag{9}$$

Differentiate (8) with respect to r.

$$\frac{\partial^2 s}{\partial r\cdot\partial t}+\frac{\partial^2\xi}{\partial r^2}+\frac{1}{r}\frac{\partial \xi}{\partial r}-\frac{\xi}{r^2}=0 \tag{10}$$

Differentiate (9) with respect to t.

$$K\frac{\partial^2 s}{\partial r\cdot\partial t}+\rho\frac{\partial^2\xi}{\partial t^2}=0 \tag{11}$$

Compare (10) and (11) and get,

$$\frac{\partial^2\xi}{\partial t^2}-\frac{K}{\rho}\left(\frac{\partial^2\xi}{\partial r^2}+\frac{1}{r}\frac{\partial \xi}{\partial r}-\frac{\xi}{r^2}\right)=0 \tag{12}$$

Suppose, $\xi=f(r)e^{ipt}$

Then, $\dfrac{\partial^2\xi}{\partial t^2}=-p^2f(r)\,e^{ipt}$ $\quad \dfrac{\partial \xi}{\partial r}=f'(r)\,e^{ipt}$

$$\frac{\partial^2\xi}{\partial r^2}=f''(r)\,e^{ipt}$$

Substitute in (12),

$$-p^2f(r)-\frac{K}{\rho}\left\{\frac{\partial^2 f}{\partial r^2}+\frac{1}{r}\frac{\partial f}{\partial r}-\frac{f}{r^2}\right\}=0 \tag{13}$$

Let $\dfrac{K}{\rho}=c^2$

and we get, $$r^2\frac{\partial^2 f}{\partial r^2}+r\frac{\partial f}{\partial r}+(k^2r^2-1)\,f=0 \tag{14}$$

where $$k^2=\frac{p^2}{c^2} \tag{15}$$

The solution of (14) is $f=AJ_1(kr)+BK_1(kr)$ (16)
Where $J_1(kr)$ = Bessel function of the first kind,
$K_1(kr)$ = Bessel function of the second kind.
Now $K_1(kr)=\infty$ for $r=0$, and therefore this solution is not admissible.
Hence full solution of Equation (12) is,

$$\xi=AJ_1(kr)e^{ipt} \tag{17}$$

Approximate boundary conditions

(a) At $r=0$, $\xi=0$. This is satisfied by Equation (17). (b) At $r=a+t$, $\xi=0$. That is, the displacement (ξ) will not quite be zero at $r=a$, due to the non-rigidity of the wall. The effect will be the same, however, as if the displacement were zero at a point just greater than $r=a$, *i.e.*, at $r=a+t$. In other words, t is a wall correction. We here assume that t, which is related to e (Part I), is constant with frequency. This simplifies the results, and the more exact boundary condition at the wall will be discussed later.

Using condition (*b*), we have,

$$\xi_{r=a+t} = \int \dot{\xi}_{r=a+t}\,dt = \frac{A}{ip}\,J_1\{k(a+t)\} = 0$$

The first root for this function occurs at $k(a+t) = 1.22\pi$

$$k = \frac{p}{c} = \frac{2\pi n}{c} \quad \text{where } n = \text{frequency.}$$

$$n = \frac{1.22\pi \times c}{2\pi(a+t)} = \frac{1.22c}{2(a+t)}$$

$a+t$ might be called the "effective radius".

Then $2(a+t)$ = "effective diameter" $= d_0$

And
$$n = \frac{1.22c}{d_0} \tag{18}$$

This is the fundamental mode of radial vibration. The higher modes will have frequencies given by the higher roots of the Bessel function. It will be noted that n is inversely proportional to the diameter of the tube.

Effect of Changing Wall Thickness

Having in mind the results obtained by applying approximate boundary conditions, we can deduce the general effect on the natural frequencies produced by changing the thickness of the tube wall.

Suppose we keep a constant, but change the thickness of the wall.

We have $n = K.\dfrac{1}{a+t}$ where K = a constant $= 1.22c$.

As the wall becomes more rigid, due to the increasing thickness, $\xi \longrightarrow 0$ at $r = a$.

And as $\xi \longrightarrow 0$ at $r = a$, $t \longrightarrow 0$.

For an absolutely rigid wall, $n_r = \dfrac{K}{a}$, where n_r = frequency for rigid wall, and $t = 0$.

In all practical cases, $t \neq 0$, though it may approach it very closely for a very thick wall. We note, however, that for thicker walls t is smaller, and hence n is greater than it is for thinner walls. That is, the frequencies of resonance are less for less rigid walls and greater for more rigid walls.

Exact Boundary Conditions

(a) At $r = 0$, $\xi = 0$. Satisfied by Equation (17). (b) At $r = a$, $\xi = e$, where ξ = displacement of the fluid at the boundary; e = change in radius of the tube wall, due to the harmonic pressure acting (see Part 1).

If ϕ is the velocity potential existing for the vibration in the liquid, we have,

$$-\frac{\partial \phi}{\partial r} = \dot{\xi}^*$$

$$\text{or } \phi = -\int \dot{\xi}\,dr$$

$$\dot{\phi} = \frac{\partial}{\partial t}\,\phi = -\frac{\partial}{\partial t}\int \dot{\xi}\,dr = -\frac{\partial}{\partial t}\int A J_1(kr) e^{ipt}\,dr$$

$$\dot{\phi} = \frac{ip}{k}\,A J_0(kr) e^{ipt} \tag{19}$$

**Reference* (2, *p. 114*)

Since $\frac{\partial}{\partial r} J_0(kr) = - kJ_1(kr)$

Now $\xi = \frac{A}{ip} J_1(kr)e^{ipt}$, found by integrating Equation (17) with respect to t.

At $r=a$, $\xi=e$.

Hence $\frac{A}{ip} J_1(ka)e^{ipt} = \frac{ipA\rho J_0(ka)e^{ipt}}{k\rho_1 h[p_0^2-p^2+\alpha^2+2i\alpha p]}$

$$\text{or } \frac{J_1(ka)}{J_0(ka)} = \frac{-p^2\rho}{k\rho_1 h[p_0^2-p^2+\alpha^2+2i\alpha p]} \tag{20}$$

The R.H.S. (right hand side) of this equation would approach ∞ at $p \longrightarrow p_0$, were it not for the resistance terms, $\alpha^2+2i\alpha p$.

Taking these into consideration, and neglecting $p_0^2-p^2$, the R.H.S. would be,

$$\frac{-p^2\rho}{k\rho_1 h(\alpha^2+2i\alpha p)}$$

of which the absolute value is, $\frac{-p^2\rho}{k\rho_1 h}\left(\frac{\sqrt{\alpha^4+4\alpha^2p^2}}{\alpha^4+4\alpha^2p^2}\right) \doteq \frac{-p^2\rho}{k\rho_1 h} \cdot \frac{1}{2\alpha p}$

$$= -\frac{p\rho}{2\alpha k\rho_1 h}, \text{ since } \alpha^4 \text{ is very small compared with } \alpha^2 p.$$

Now $k = \frac{p}{c}$

Hence at $p=p_0$, R.H.S. $\doteq -\frac{p\rho c}{2\alpha\rho_1 hp} = -\frac{\rho c}{2\alpha\rho_1 h}$.

Now the numerator of this fraction is very much greater than the denominator. Hence the R.H.S. is still very great when $p \longrightarrow p_0$, though not $\longrightarrow \infty$.

Since the curve of $\frac{J_1(x)}{J_0(x)}$ against x is very steep at the point where $\frac{J_1(x)}{J_0(x)} \longrightarrow \infty$, it does not change the value of x by any appreciable amount whether we let the R.H.S. of Equation (20) $=\infty$ or only a large number (say >50).

Hence at $p=p_0$, we can neglect the part of the denominator of the R.H.S. of Equation (20) represented by the terms, $\alpha^2+2i\alpha p$.

It is only in the neighborhood of $p=p_0$ that the terms $\alpha^2+2i\alpha p$ become of importance compared with $p_0^2-p^2$.

We have shown that the value of x (ka in our equation) is not affected by changes in the denominator when the denominator is small (*i.e.*, in the neighborhood of $p=p_0$). Since only in this neighborhood do the resistance terms become important, and since here they make no appreciable difference, we can neglect these terms for all cases, and have only,

$$\text{R.H.S.} = \frac{-p^2\rho}{k\rho_1 h(p_0^2-p^2)}$$

Equation (20) now becomes

$$\frac{J_1(ka)}{J_0(ka)} = \frac{-p^2\rho}{k\rho_1 h(p_0^2-p^2)} = \frac{-p^2\rho}{k\rho_1 h\left(\frac{E^1}{\rho_1 a^2}-p^2\right)} = \frac{-p^2\rho}{k\left(\frac{E^1 h}{a^2}-p^2\rho_1 h\right)} \tag{21}$$

But $k = \frac{p}{c}$ or $p=kc$

Therefore,

$$\text{R.H.S.} = \frac{-k^2c^2\rho}{k\left(\frac{E^1h}{a^2} - k^2c^2\rho_1 h\right)} = \frac{-(ka)c^2a\rho}{h[E^1 - k^2a^2c^2\rho_1]}$$

Let $ka = x$. Then for Equation (21) we have,

$$\frac{J_1(x)}{J_0(x)} = \frac{-xac^2\rho}{h[E^1 - x^2c^2\rho_1]} \tag{22}$$

From Equation (22) we can determine x, which in turn determines the resonant frequency for radial vibrations in the tube, by the following relation,—

$$ka = \frac{p_0 a}{c} = x \qquad \text{or} \qquad p_0 = \frac{cx}{a}$$

$$n = \frac{p_0}{2\pi} = \frac{c_1 x}{2\pi a} \tag{23}$$

Remarks

Equation (23) is, $\quad n = \dfrac{\frac{x}{\pi}\cdot c}{2a}$

Compare this with the result obtained by approximating the boundary conditions,

$$n = \frac{1.22c}{2(a+t)} \tag{18}$$

We now have a variable $\dfrac{x}{\pi}$ compared with the constant 1.22; and we use the real diameter $2a$ instead of the "effective diameter" $\{d_0 = 2(a+t)\}$, but the form of the equation is unaltered.

The reason for the variable $\dfrac{x}{\pi}$ is that the resonant frequency (n_0) changes with changing boundary conditions (varying t), and we saw from a consideration of the exact boundary conditions that these conditions are dependent upon frequency.

Part III. Transmission of Sound in Liquids Contained in Cylindrical Tubes. Vibrations Both Longitudinally and Radially

Consider the velocity potential, ϕ, which exists for all cases of fluid motion when no circulation exists. This is obviously the case in sound.

We have the expression, $\quad \ddot{\phi} - c^2\nabla^2\phi = 0$* $\qquad$ (24)

Where $c = \sqrt{\dfrac{K}{\rho}}$ = velocity in the unconfined liquid.

In cylindrical co-ordinates, if ϕ is independent of θ, which is true in purely longitudinal and radial vibrations, we have,

$$\nabla^2\theta = \frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial\phi}{\partial r}\right) + \frac{\partial^2\phi}{dz^2} \tag{25}$$

Substitute in (24),

$$\frac{\partial^2\phi}{\partial t^2} - c^2\left(\frac{\partial^2\phi}{\partial r^2} + \frac{1}{r}\frac{\partial\phi}{\partial r} + \frac{\partial^2\phi}{\partial z^2}\right) = 0 \tag{26}$$

**Reference (2) p. 116, Equation 151a.*

Suppose ϕ is of the form, $\phi = A\, f(r)\, e^{i\omega\left(t-\frac{z}{c_1}\right)}$ where c_1 = phase velocity.

Substitute in (26),

$$-\omega^2 f - c^2\left(\frac{\partial^2 f}{\partial r^2} + \frac{1}{r}\frac{\partial f}{\partial r} - \frac{\omega^2}{c_1^2} f\right) = 0$$

$$\text{or, } \frac{\partial^2 f}{\partial r^2} + \frac{1}{r}\frac{\partial f}{\partial r} + \omega^2\left(\frac{1}{c^2} - \frac{1}{c_1^2}\right) f = 0 \tag{27}$$

Write as, $$\frac{\partial^2 f}{\partial r_2} + \frac{1}{r}\frac{\partial f}{\partial r} + k^2 f = 0 \tag{28}$$

Where, $$k^2 = \omega^2\left(\frac{1}{c^2} - \frac{1}{c_1^2}\right) \tag{29}$$

For k to be real, $\frac{1}{c^2} > \frac{1}{c_1^2}$ or $c_1 > c$. If $c_1 < c$, $\frac{1}{c_1^2} > \frac{1}{c^2}$.

And $k^2 = -\omega^2\left(\frac{1}{c_1^2} - \frac{1}{c^2}\right)$

Or $k = i\alpha$, where $$\alpha = \omega\sqrt{\frac{1}{c_1^2} - \frac{1}{c^2}} \tag{30}$$

Hence we have two cases, (A) k real, (B) k imaginary $= i\alpha$.

Case (A)

If k be real, the solution of (28) is, $f = A\,J_0(kr)$

And hence, $$\phi = A\,J_0(kr)e^{i\omega\left(t-\frac{z}{c_1}\right)} \tag{31}$$

Let $\dot\zeta$ = particle velocity in r direction.
$\dot\xi$ = particle velocity in z direction.

$$\dot\zeta = -\frac{\partial\phi}{\partial r} = +kA\,J_1(kr)\,e^{i\omega\left(t-\frac{z}{c_1}\right)} \tag{32}$$

And $$\zeta = \int \dot\zeta\, dt = \frac{Ak}{i\omega} J_1(kr)\, e^{i\omega\left(t-\frac{z}{c_1}\right)} \tag{33}$$

Approximate Boundary Conditions for Case (A)

$\zeta = 0$ at $r = 0$, and at $r = a+t$, where a = radius of cylinder, $a+t$ = "effective radius".

These conditions are satisfied if, $J_1\{k(a+t)\} = 0$

$J_1\{k(a+t)\} = 0$, when $k(a+t) = 1.22\pi$ (1st mode) or $k = \dfrac{1.22\pi}{a+t}$

But $k = \omega\sqrt{\frac{1}{c^2} - \frac{1}{c_1^2}}$, and $\omega = 2\pi n$, where n = frequency.

Therefore, $$\frac{1}{c^2} - \frac{1}{c_1^2} = \frac{(1.22)^2\pi^2}{(a+t)^2 4\pi^2 n^2} \tag{34}$$

Which leads to, $$c_1 = \frac{nc}{\sqrt{n^2 - n_0^2}} \tag{35}$$

Where $n_0 = \dfrac{1.22c}{2(a+t)}$ = fundamental mode of the radial vibration [see Equation (18)].

The form of the curve resulting from Equation (35) is shown in Fig. 4.

Case (B)

k imaginary $(=i\alpha)$.

Equation (28) is now,

$$\frac{\partial^2 f}{\partial r^2} + \frac{1}{r}\frac{\partial f}{\partial r} - \alpha^2 f = 0 \tag{36}$$

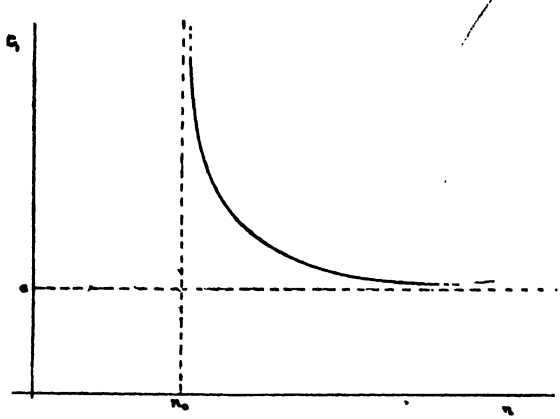


FIG. 4. *Curve of velocity against frequency after first absorption band, using approximate boundary conditions.*

Of which the solution is, $f = I_0(\alpha r)$

Where $I_0(\alpha r) = J_0(i\alpha r) = J_0(kr)$

Hence,
$$\phi = A I_0(\alpha r) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{37}$$

$$\dot{\zeta} = -\frac{\partial \phi}{\partial r} = -\alpha I_1(\alpha r) A e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{38}$$

$$\zeta = -\int \frac{\partial \phi}{\partial r}\cdot dt = \frac{-\alpha}{i\omega} I_1(\alpha r) A e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{39}$$

Approximate Boundary Conditions for Case (B)

$\zeta = 0$ at $r = 0$ and at $r = a + t$.

Now $I_1(\alpha r) = 0$ for $\alpha r = 0$ and *for no other value* of αr. $\alpha r = 0$ at $r = 0$.

$\alpha r \neq 0$ at $r = a + t$ unless $\alpha = 0$. *i.e.*, $\omega\sqrt{\dfrac{1}{c_1^2} - \dfrac{1}{c^2}} = 0$

$$\omega\sqrt{\frac{1}{c_1^2} - \frac{1}{c^2}} = 0 \text{ if } c_1 = c.$$

This means that when the argument is imaginary, it is zero for all frequencies, and $c_1 = c$. In other words, we have the following situation.

For $n > n_0$, where n_0 is the absorbing frequency, the wave velocity is,

$$c_1 = \frac{cn}{\sqrt{n^2 - n_0^{\,2}}}$$

For $n < n_0$, the wave velocity is constant and given by, $c_1 = c$.

This is obviously only a first approximation, as a diminished wave velocity due to lateral expansion of the tube wall is not indicated anywhere in this solution.

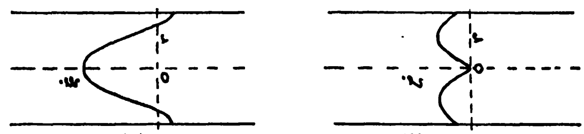
Particle Velocities for Case (A) and Case (B)

Case (A) $n > n_0$

$$\dot{\xi} = -\frac{\partial \phi}{\partial z} = \frac{+i\omega}{c_1} A J_0(kr) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{40}$$

This becomes zero for $kr = 0.766\pi$, *i.e.*, for $r = \dfrac{0.766}{1.22}(a+t)$

And after that it reverses in sign. See Fig. 5.



(a) (b)

FIG. 5. *Particle velocities after first absorption band. (a) Longitudinal velocity; (b) radial velocity.*

$$\dot{\zeta} = -\frac{\partial \phi}{\partial r} = kA J_1(kr) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{41}$$

Note that $\dot{\xi}$ is 90° out of phase with $\dot{\zeta}$, due to the factor $+i$ by which $\dot{\xi}$ is multiplied. For $n \rightarrow n_0$, $c_1 \rightarrow \infty$, and therefore,

$$\dot{\xi}\left(= \frac{i\omega}{c_1} A J_0(kr) e^{i\omega\left(t - \frac{z}{c_1}\right)}\right) \rightarrow 0.$$

This means that for the frequency of radial resonance the wave is propagated with difficulty longitudinally (in the z-direction); that is, there is a great deal of absorption. Also note that the longitudinal wave is not plane.

Case (B)

$$\phi = A I_0(\alpha r) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{37}$$

Here $\alpha = 0$, and since $I_0(0) = 1$, we have, $\phi = A e^{i\omega\left(t - \frac{z}{c_1}\right)}$

$$\dot{\xi} = -\frac{\partial \phi}{\partial z} = \frac{i\omega}{c_1} A e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{42}$$

$\dot{\xi}$ is independent of r — it is a plane wave. This is an approximation, true only for low frequencies, as will be discussed later.

Also
$$\dot{\zeta} = -\frac{\partial \phi}{\partial r} = 0 \tag{43}$$

Exact Boundary Conditions Applied

Case (A) k real.

$$\phi = A J_0(kr) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{31}$$

$$\dot{\zeta} = kA J_1(kr) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{32}$$

$$\zeta = \frac{Ak}{i\omega} J_1(kr) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{33}$$

The conditions are,

(i) At $r=0$, $\zeta=0$; satisfied by Equation (33).

(ii) At $r=a$, $\zeta=$e.

Where $e = \dfrac{\rho\phi}{\rho_1 h[p_0^2 - \omega^2]}$ (5)

And in which expression we neglect terms involving the damping factor (α) for vibration in the tube wall.

Also, $$\phi = Ai\omega J_0(kr) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{44}$$

Hence Condition (ii) leads to,

$$\frac{Ak}{i\omega} J_1(ka) e^{i\omega\left(t - \frac{z}{c_1}\right)} = \frac{\rho Ai\omega J_0(ka)\, e^{i\omega\left(t - \frac{z}{c_1}\right)}}{\rho_1 h[p_0^2 - \omega^2]} \tag{45}$$

or, $$\frac{J_1(ka)}{J_0(ka)} = \frac{-\rho\omega^2}{k\rho_1 h[p_0^2 - \omega^2]} \tag{46}$$

Recall that $p_0^2 = \dfrac{E^1}{\rho_1} \cdot \dfrac{1}{a^2}$ (4)

We now have,

$$\text{R.H.S.} = \frac{-\rho\omega^2}{k\rho_1 h\left[\frac{E^1}{\rho_1 a^2} - \omega^2\right]} = \frac{-a}{kah\left[\frac{E^1}{a^2\omega^2\rho} - \frac{\rho_1}{\rho}\right]}$$

From (46) we then get, writing $ka = x$,

$$\frac{J_1(x)}{J_0(x)} = -\frac{a}{h}\,\frac{1}{x\left[\frac{E^1}{a^2\omega^2\rho} - \frac{\rho_1}{\rho}\right]} \tag{47}$$

Or, $$x\frac{J_1(x)}{J_0(x)} = \frac{-\frac{a}{h}}{\frac{E^1}{a^2\omega^2\rho} - \frac{\rho_1}{\rho}} \tag{48}$$

Now, $$x = ka = a\omega\sqrt{\frac{1}{c^2} - \frac{1}{c_1^2}}$$

And hence, $c_1^2 = \dfrac{c^2\omega^2}{\omega^2 - \frac{c^2x^2}{a^2}}$ (49)

Or, since $\omega = 2\pi n$,

$$c_1^2 = \frac{n^2c^2}{n^2 - \frac{x^2c^2}{4\pi^2a^2}} \tag{50}$$

Let $$n_x = \frac{\frac{x}{\pi}\,.c}{2a} \tag{51}$$

Then, $$c_1 = \frac{nc}{\sqrt{n^2 - n_x^2}} \tag{52}$$

By solving (48) we obtain x, and from (52) we then obtain c_1.

Notice the resemblance between the results obtained for the approximate boundary conditions and those obtained for the exact boundary conditions. The difference is that in the equation for n_x we have a variable $\frac{x}{\pi}$ instead of the constant (1.22) previously obtained for n_0. This occurs because the change in radius of the tube (e) is not constant, but varies with frequency; and therefore the resonant frequency (n_x) for radial vibrations in the liquid also changes.

Case (B) k imaginary.

$$\phi = A I_0(\alpha r) e^{i\omega\left(t - \frac{z}{c^1}\right)} \tag{37}$$

$$\dot{\zeta} = -\frac{\partial \phi}{\partial r} = -\alpha I_1(\alpha r) A e^{i\omega\left(t - \frac{z}{c^1}\right)} \tag{53}$$

$$\zeta = \int \dot{\zeta} dt = -\frac{\alpha}{i\omega} I_1(\alpha r) A e^{i\omega\left(t - \frac{z}{c^1}\right)} \tag{54}$$

$$\dot{\phi} = i\omega A I_0(\alpha r) e^{i\omega\left(t - \frac{z}{c^1}\right)} \tag{55}$$

The conditions are,

(i) At $r=0$, $\zeta=0$; satisfied by Equation (54).

(ii) At $r=a$, $\zeta=e$, where e is as defined under Case (A).

From Condition (ii) we have,

$$-\frac{\alpha}{i\omega} I_1(\alpha a) A e^{i\omega\left(t - \frac{z}{c^1}\right)} = \frac{\rho i\omega A I_0(\alpha a) e^{i\omega\left(t - \frac{z}{c^1}\right)}}{\rho_1 h[p_0^2 - \omega^2]} \tag{56}$$

Or,
$$\alpha \frac{I_1(\alpha a)}{I_0(\alpha a)} = \frac{\rho \omega^2}{\rho_1 h[p_0^2 - \omega^2]} \tag{57}$$

Using a somewhat different method of derivation, Korteweg (3) arrived at a result equivalent to Equation (57), and from it obtained his expression for phase velocity. He seems, however, not to have considered the possibility of the argument becoming real ($c_1 > c$), and so missed the second solution represented by Case A.

Now
$$p_0^2 = \frac{E^1}{\rho_1 a^2}$$

Hence we have, from (57),

$$\frac{\alpha a I_1(\alpha a)}{I_0(\alpha a)} = \frac{a\rho\omega^2}{h\rho_1\left[\frac{E^1}{\rho_1 a^2} - \omega^2\right]} = \frac{\frac{a}{h}}{\frac{E^1}{a^2\omega^2\rho} - \frac{\rho_1}{\rho}} \tag{58}$$

Or,
$$x^1 \frac{I_1(x^1)}{I_0(x^1)} = \frac{\frac{a}{h}}{\frac{E^1}{a^2\omega^2\rho} - \frac{\rho_1}{\rho}} \tag{59}$$

Now,
$$x^1 = \alpha a = a\omega\sqrt{\frac{1}{c_1^2} - \frac{1}{c^2}} \tag{30a}$$

And,
$$\omega = 2\pi n$$

Therefore,
$$c_1 = \frac{cn}{\sqrt{n^2 + n^2_{x^1}}} \tag{60}$$

Where,
$$n_{x^1} = \frac{\frac{x^1}{\pi} \cdot c}{2a} \tag{61}$$

Equation (59) is true for all values of ω and x^1, providing x^1 exists and $c_1 < c$, and it gives us an equation for determining x^1 and hence c_1.

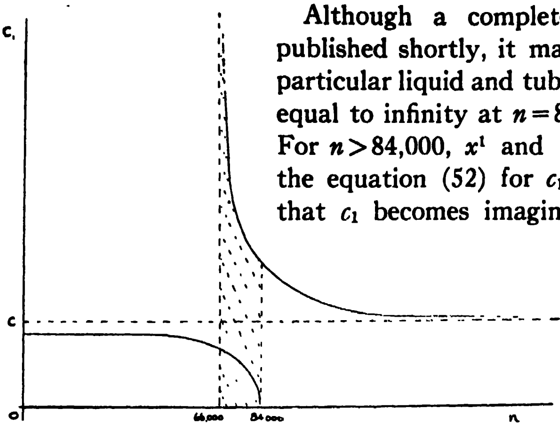


FIG. 6. *Complete velocity-frequency curve for a particular case where the absorbing frequency equals 65000.*

Although a complete experimental paper will be published shortly, it may be mentioned here that for a particular liquid and tube, Case *B* shows that x^1 becomes equal to infinity at $n = 84{,}000$, and at this point $c_1 = 0$. For $n > 84{,}000$, x^1 and hence c_1 are imaginary. From the equation (52) for c_1 in Case *A*, however, we find that c_1 becomes imaginary for $n < 65{,}000$. Hence we see that there is a range (for frequencies between 65,000 and 84,000) where both types of vibration tend to exist. In this range, therefore, both vibrations will be unstable and it will be extremely difficult to measure wave velocities for these frequencies.

The theoretical velocity-frequency curve is shown in Fig. 6. The theoretical frequency of radial resonance for the case mentioned is found from Equations 22 and 23 to be 65,000 cycles. The shaded area shows the region where vibrations are unstable.

Approximation for Low Frequencies

For low frequencies we are obviously dealing with Case *B*.

We had,
$$\alpha a = a\omega\sqrt{\frac{1}{c_1^2} - \frac{1}{c^2}} = \frac{a\omega}{c_1}\sqrt{1 - \frac{c_1^2}{c^2}} \tag{30a}$$

Or,
$$\alpha a = \frac{2\pi a}{\lambda}\sqrt{1 - \frac{c_1^2}{c^2}} \quad \text{where } \lambda = \text{wave-length.}$$

If the frequency be low, $\frac{2\pi a}{\lambda}$ is a small fraction, and we can develop $I_0(\alpha a)$ and $I_1(\alpha a)$ by using only two terms of the series expansion, *i.e.*,

$$I_0(\alpha a) \doteq 1 + \frac{\alpha^2 a^2}{4} = 1 + \frac{{x^1}^2}{4} \tag{62}$$

$$I_1(\alpha a) \doteq \frac{\alpha a}{2}\left(1 + \frac{\alpha^2 a^2}{8}\right) = \frac{x^1}{2}\left(1 + \frac{{x^1}^2}{8}\right) \tag{63}$$

Substituting (62) and (63) in (59), we get,

$$\frac{\frac{{x^1}^2}{2}\left(1 + \frac{{x^1}^2}{8}\right)}{1 + \frac{{x^1}^2}{4}} = \frac{\frac{a}{h}}{\frac{E^1}{a^2\omega^2\rho} - \frac{\rho_1}{\rho}} \tag{64}$$

Which gives, $\frac{1+x^{1^2}}{4} = \left(\frac{hE^1}{2\rho a} - \frac{h\rho_1 a\omega^2}{2\rho}\right)\left(\frac{1}{c_1^2} - \frac{1}{c^2}\right)\left(1+\frac{x^{1^2}}{8}\right)$ (65)

Since $x^{1^2} = a^2\omega^2\left(\frac{1}{c_1^2} - \frac{1}{c^2}\right)$

But, $1+\frac{x^{1^2}}{4} \doteq \left(1+\frac{x^{1^2}}{8}\right)^2$, neglecting $\frac{x^{1^4}}{64}$

Hence we have,

$$1+\frac{x^{1^2}}{8} = \frac{hE^1}{2\rho a}\left(\frac{1}{c_1^2} - \frac{1}{c^2}\right)\left(1-\frac{\rho_1 a^2}{E^1}\omega^2\right) \tag{66}$$

And finally,

$$c_1^2 = \left(1 - \frac{c_1^2}{c^2}\right)\frac{h}{a}\left\{\frac{E^1}{2\rho} - \frac{\rho_1 a^2 2\pi^2 n^2}{\rho} - \frac{\pi^2 a^2 n^2}{2}\right\} \tag{67}$$

For ordinary glass, $E^1 = 6.78 \times 10^{11}$.
If $n = 5{,}000$, $n^2 = 2.5 \times 10^7$.
Hence for low frequencies we neglect the second and third terms in the bracket on the R.H.S. of (67) compared with the first term.

Then, $$c_1^2 = \left(1 - \frac{c_1^2}{c^2}\right)\frac{hE^1}{2\rho a} \tag{68}$$

Or, $$c_1^2 = \frac{c^2}{\frac{2\rho a c^2}{hE^1} + 1} \tag{69}$$

And since, $c^2 = \frac{K}{\rho}$

We have, $$c_1^2 = \frac{c^2}{1+\frac{2Ka}{hE_1}} \tag{70}$$

This is the expression given by Korteweg (3) and Lamb (4, p. 177, Equation 12; 5, p. 1), as their simple formula.

Taking a particular liquid and tube (the same as previously mentioned), and working out wave velocities from Equations 59 and 60 and from Equation 70, we find the velocity in both cases equal to 1.14×10^5cm./sec. at 1000 cycles. Hence the accurate and approximate formulas give the same results (to at least 1%) at that frequency. At 20,000 cycles there is a slight deviation, as Equations 59 and 60 give a velocity of 1.13×10^5 cm./sec., while Equation 70 gives, of course, the same result as before (since it is independent of frequency). Hence the lateral movement of the tube wall causes a diminution in wave velocity which is practically constant for low frequencies.

An interesting result from the foregoing investigation is that above the absorbing frequency the phase velocity is always greater than the unconfined wave velocity, approaching it asymptotically as the frequency increases. For very high frequencies (very short wave-lengths), the velocity would be practically equal to the value in an infinite medium, however, which is what we should expect.

Particle Velocities

Case A $c_1 > c$

$$\phi = A J_0(kr) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{31}$$

Here the expression for ϕ is the same as was discussed under "Approximate Boundary Conditions". The particle velocities, therefore, are the same as were treated there. It should be added, however, that if we take for an example the same tube and liquid mentioned above, we find that the particle velocity ($\dot{\xi}$) becomes zero at $r = a$ for a frequency of 84,000. As the frequency is then lowered to 65,000, the node of particle velocity moves nearer to the axis, with the result that in the centre of the tube the particle velocity ($\dot{\xi}$) is 180° out of phase with that in the annulus between the node and the wall of the tube. The situation is then as is shown in Fig. 5. Above 84,000 cycles, $\dot{\xi}$ is not zero for any value of a.

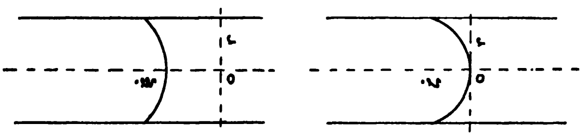
Case B $c_1 < c$

$$\phi = A I_0(\alpha r) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{37}$$

$$\dot{\xi} = -\frac{\partial \phi}{\partial z} = +\frac{i\omega}{c_1} A I_0(\alpha r) e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{71}$$

$$\dot{\zeta} = -\frac{\partial \phi}{\partial r} = -\alpha I_1(\alpha r) A e^{i\omega\left(t - \frac{z}{c_1}\right)} \tag{53}$$

Fig. 7 shows the way the particle velocities vary with r, *i.e.*, distance from the axis of the tube.



(*a*) (*b*)

FIG. 7. *Particle velocities before first absorption band.* (*a*) *Longitudinal velocity;* (*b*) *radial velocity.*

Note that $\dot{\zeta}$ rises from zero at $r = 0$ to a maximum at $r = a$. The particle velocity $\dot{\xi}$ is a minimum at $r = 0$ and increases to a maximum at $r = a$.

For the liquid and tube previously mentioned, if $\dot{\xi}$ at $r = 0$ be represented by unity, then at $r = a$, we have particle velocities as shown in Table I.

TABLE I

PARTICLE VELOCITIES AT WALL OF TUBE FOR VARIOUS FREQUENCIES

n	20000	30000	40000	50000	60000	70000
$\dot{\xi}$	1.06	1.17	1.39	2.0	4.2	32.6

These values mean that for audible frequencies (below 20000) the longitudinal wave is sensibly plane, since the velocity at the side of the tube is approximately the same as at the centre. As the frequency approaches the absorbing frequency, however, the wave departs from its plane form; the particle velocity at the boundary tends to become much greater than at the centre.

In the foregoing, viscosity is not taken into account, which will of course tend to reduce the velocity at the wall of the tube, so that the wave will not depart from its plane form as much as would be indicated by our equations.

Since decreased phase velocity in the neighborhood of the absorbing frequency goes hand in hand with increased $\dot{\xi}$ at the wall of the tube, if $\dot{\xi}$, due to viscosity, is prevented from increasing to some extent, then the phase velocity (c_1) will not be diminished as much as would be indicated by our equations.

Higher Modes of Radial Vibration

Although only the fundamental mode of radial vibration has been discussed so far, it is obvious that the higher modes, given by the higher roots of Equation (22), require some mention.

For the tube and liquid already considered, it is found that the second mode occurs at 102,000 cycles. Since the functions $I_0(x^1)$ and $I_1(x^1)$ are not periodic, the type of vibration represented by Case B cannot exist except in the range 0 to 84,000 cycles. Above the latter frequency, therefore, it is only Case A which need be considered.

If the second mode of radial vibration does not occur, the phase velocity will continue to approach the unconfined value asymptotically. If, however, it does occur, the velocity will be the same as above up to the resonant frequency. At that frequency, the occurrence of the second mode will tend to force it to a very high value again, as did the fundamental vibration at its resonant frequency. Above this frequency, the velocity will again approach the unconfined value, until the third mode is reached, when the situation will be repeated.

There is, however, a practical consideration which will tend to prevent the higher modes from occurring, except for comparatively low fundamentals. We saw, in considering particle velocities, that near the frequency of the fundamental mode the particle velocity ($\dot{\xi}$) reversed in phase at a certain distance from the axis of the tube. For the second mode of vibration, there will be two reversals between $r=0$ and $r=a$. For the third mode, three reversals will occur, and so on. Each additional reversal of phase will cause the particles of liquid moving with opposite phase to become closer together. Viscosity will enter into the question, and it is to be expected that these nodes will have to be a certain minimum distance from each other.

Now, the narrower the tube the closer together will the particles moving with opposite phase have to be for a given number of reversals. That is, for a certain wide tube, the requisite minimum distance of one reversal from the other (node to node distance) will allow, say, three of these nodes to occur; in other words, three modes of radial vibration can exist when a longitudinal wave is propagated along the tube. Since these modes are inversely proportional to the diameter of the tube, and the tube is wide, the third mode may have a frequency no higher than the second or even the fundamental mode for a tube that is considerably narrower. With the narrow tube, then, it may be that only the first or second mode can occur, as higher modes would necessitate the nodes of $\dot{\xi}$ to be closer together than the minimum distance.

The foregoing paragraph may be summarized by saying that for any liquid there will be a maximum frequency above which resonant radial vibrations cannot occur, as viscosity will prevent particles very close together from moving with opposite phase.

Acknowledgment

The writer wishes to thank Dr. R. Ruedy, National Research Laboratories, who obtained for him an abstract of Korteweg's (3) paper, and who checked most of the mathematics.

References

1. Boyle, R. W. and Froman, D. Nature, 126: 602-603. 1930.
2. Crandall, I. B. Theory of vibrating systems and sound. Van Nostrand. 1926.
3. Korteweg, D. J. Ann. Physik, 5: 525-542. 1878.
4. Lamb, H. Dynamical theory of sound. 2d. ed. E. Arnold. 1925.
5. Lamb, H. Mem. Manchester Phil. and Lit. Soc. 42, No. 9. 1898.

ON THE PROPAGATION OF LONGITUDINAL WAVES IN CYLINDRICAL RODS[1]

By R. Ruedy[2]

Abstract

The solution of the velocity equation obtained by Pochhammer on the basis of the mathematical theory of elasticity is determined for the propagation of longitudinal waves of any frequency in a long solid circular cylinder of any diameter. For a given frequency a large number of solutions may be obtained, but when the condition is imposed that for low frequencies the velocity must gradually assume the value found by experiment, a single value is obtained for each frequency. The velocity decreases with increasing frequency, so that, for a cylinder of finite length, the resonance frequencies come closer and closer together. It is also necessary to take into account, however, that in a solid rod longitudinal waves are accompanied by radial vibrations of the particles, and that a cylindrical rod has, regardless of its length, a series of natural frequencies for radial waves, so that for wave-lengths comparable with the diameter of the tube a coupled system of oscillations is set up. The resonant frequencies of such a system depend on the degree of coupling.

Introduction

High mechanical frequencies of vibration produced by taking advantage of piezo-electric effects promise to become an effective tool of investigation. Due to the small wave-length, high frequency vibrations allow acoustic effects to be studied within the confines of the ordinary laboratory. Given strong sources of high frequency waves the metallurgist and the engineer are able to run hysteresis and endurance tests in a much shorter time, even when they have to extend them to several hundred million cycles. As, on the other hand, in the piezo-electric effect, for instance, definite atoms of a group are set in vibration with respect to the rest of the group, a certain amount of chemical activation is to be expected and an influence upon the rate of chemical reaction might be found.

Many of the quantitative expressions given for the propagation and absorption of sound waves in different simple structures, such as rods, tubes or plates, are only valid for waves of moderate frequencies. In view of the simple fact that quartz slabs and bars are now used for maintaining oscillations of over one million cycles per second, constant to within less than fifty cycles, it is desirable to extend the velocity formulas to very high frequencies. As a beginning, the velocity of propagation of the waves in a long circular cylinder was calculated, because this is a simple case and because unpublished work by Boyle seems to show that a discontinuity exists at a certain high frequency.

The Equation for the Velocity of Propagation of Longitudinal Waves

The elementary theory valid for long waves assumes that all the points of a perpendicular cross section are not only in phase, but have the same swinging motion along the axis of the cylinder. On this assumption the square of the

1 *Manuscript received June 2, 1931.*

Contribution from the National Research Laboratories, Ottawa. This paper was read before Section III of the Royal Society of Canada in May, 1931.

2 *Junior Research Investigator, National Research Laboratories, Ottawa.*

velocity of propagation of longitudinal waves becomes equal to E/d, where E is Young's modulus and d is the density of the material. As, however, any section of the rod which is expanding along the axis is contracted along the diameter, there arises at the same time a motion of the particles in the direction of the radius. This causes a passing radial increase in density, and therefore, a decrease in the velocity of sound. In the case of uniform elastic solid cylinders the theory gives the following general expression for the propagation of mechanical vibrations (5):

$$\left(2G\,\frac{\partial J_0(h'a)}{\partial a^2} - \frac{p^2 d}{m-1}J_0(h'a)\right)\left(2-\frac{c^2 d}{G}\right) = 4G\,\frac{\partial J_0(h'a)}{\partial a}\,\frac{\partial J_1(k'a)}{\partial a}\Big/ J_1(k'a)$$

where G = modulus of elasticity in shear, $p = 2\pi$ times the frequency f, a = radius of cylinder, c = velocity of propagation of longitudinal waves, $c_0^2 = E/d$,

$$h'^2 = \frac{p^2}{c^2}\left(\frac{c^2}{c_0^2}\,\frac{(m-2)(m+1)}{(m-1)m} - 1\right) = \frac{p^2}{c^2}H'^2, \qquad k'^2 = \frac{p^2}{c^2}\left(\frac{c^2}{c_0^2}\,\frac{2(m+1)}{m} - 1\right) = \frac{p^2}{c^2}K'^2$$

and $2 < m < 5$. The letter m designates the reciprocal of Poisson's ratio; J_0 and J_1 are Bessel's functions of the first kind, of order zero and one. When the assumption is made that f as well as a is very small, the series development of the functions may be stopped after the first or second term, and this leads to the same expressions as are given by the elementary theory (4). For discussing the general case, a more distinct form must be sought for the equation. Replacing the modulus of elasticity in shear by Young's modulus according to the formula $G = \frac{mE}{2(m+1)}$ we get

$$\left(1 - \frac{c^2}{c_0^2}\,\frac{m+1}{m}\right)\left(\frac{J_1(h'a)}{h'aJ_0(h'a)} - \frac{\frac{c^2}{c_0^2}\,\frac{(m+1)}{m} - 1}{\frac{c^2}{c_0^2}\,\frac{(m-2)(m+1)}{(m-1)m} - 1}\right) = \frac{J_1(h'a)}{h'aJ_0(h'a)}\left(1 - \frac{k'aJ_0(k'a)}{J_1(k'a)}\right)$$

This equation depends essentially on the ratio c/c_0. Experiment shows that for low frequencies c is smaller than c_0 so that H^2 becomes negative. Writing therefore $ih = h'$ or $H^2 = -H'^2 = 1 - \frac{c^2}{c_0^2}\,\frac{(m-2)(m+1)}{(m-1)\;m}$ the equation determining the velocity of propagation becomes

$$\left(1 - \frac{c^2}{c_0^2}\,\frac{m+1}{m}\right)\left(\frac{iJ_1(iha)}{haJ_0(iha)} + \frac{\frac{c^2}{c_0^2}\,\frac{(m+1)}{m} - 1}{\frac{c^2}{c_0^2}\,\frac{(m-2)(m+1)}{(m-1)m} - 1}\right) = \frac{iJ_1(iha)}{haJ_0(iha)}\left(1 - \frac{\kappa haJ_0(\kappa ha)}{J_1(\kappa ha)}\right)$$

$$\text{with } \kappa = \frac{K'}{H} = \sqrt{\frac{\frac{c^2}{c_0^2}\,\frac{2(m+1)}{m} - 1}{1 - \frac{c^2}{c_0^2}\,\frac{(m-2)(m+1)}{(m-1)m}}}$$

$$ha = \frac{p}{c}Ha$$

The values for the fractions containing Bessel functions are represented in the graph. Restricting for the moment the considerations to the case where h and k' are real and positive, and taking into account that the values for m

vary between from more than 2 to not quite 5, the main features of the solution may be recognized.

1. The solution does not depend on the sign of h; it is therefore sufficient to take the root of H^2 with its positive sign throughout.

2. For values of ha and $k'a$ approaching zero, that is, for very low frequencies, or for very thin cylinders, the equation is satisfied when $c=c_0$. There is no other value for which this happens.

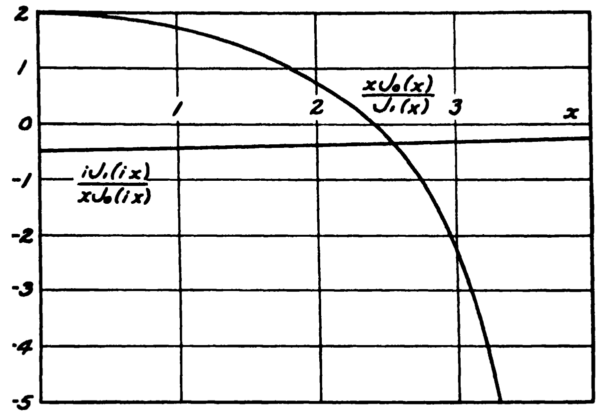


FIG. 1. *Functions used in velocity equation.*

3. The first factor on the left hand side is negative for $c=c_0$, increases to more positive values when c decreases and becomes equal to zero for

$$\frac{c^2}{c_0^2} = \frac{m}{m+1} < 1$$

On the left hand side evidently $1=\frac{(\kappa ha)J_0(\kappa ha)}{J_1(\kappa ha)}$ or, from the graph $ha\,\kappa \approx 1.85$ and at the same time $\kappa=\sqrt{m-1}$; for instance, for

	$m =$	8	5	4	3	2.5
	$c/c_0 =$	0.94	0.91	0.89	0.87	0.85
	$\kappa =$	2.64	2	1.73	1.41	1.23
	$ha =$	0.70	0.93	1.06	1.31	1.50
therefore	$f \approx$	$0.27\frac{c_0}{a}$	$0.27\frac{c_0}{a}$	$0.26\frac{c_0}{a}$	$0.25\frac{c_0}{a}$	$0.27\frac{c_0}{a}$

4. The first term in the second bracket on the left hand side is always negative and varies from -0.5 to $-\infty$ when the independent variable ha varies from zero to infinity. The second term, which is negative for the long waves, the denominator being equal to H'^2, becomes positive for values of $\frac{c^2}{c_0^2} < \frac{m}{m+1} < 1$ and the entire left hand side becomes once more equal to zero when

$$\frac{iJ_1(iha)}{haJ_0(iha)} = \frac{\frac{c^2}{c_0^2}\frac{(m+1)}{m} - 1}{\frac{c^2}{c_0^2}\frac{(m-2)\,(m+1)}{(m-1)m} - 1}$$

The value of κha on the right hand side will again be equal to about 1.85, but the value of ha will be larger than before due to the circumstance that κ decreases when c/c_0 drops to lower values. The exact solution must be found by trial, but the main result is that c/c_0 decreases to below $m/(m+1)$.

5. The lowest velocity for which the equation as written admits of a solution is $\frac{c^2}{c_0^2} = \frac{m}{2(m+1)}$ when K' becomes equal to zero and the values of ha are for

m =	8	5	4	3	2.5
c/c_0 =	0.67	0.65	0.63	0.62	0.6
c_0h =	$7.1f$	$7.7f$	$8.1f$	$8.8f$	$9.6f$

or, as from the velocity equation for $k' = 0$ and $h \neq 0$, $\frac{iJ_1(iha)}{haJ_0(iha)} = \frac{1-m}{3m}$,

or ha ≈	2.7	3.0	3.3	3.8	4.3
and therefore f ≈	$0.38\frac{c_0}{a}$	$0.39\frac{c_0}{a}$	$0.4\frac{c_0}{a}$	$0.43\frac{c_0}{a}$	$0.45\frac{c_0}{a}$

When c/c_0 continues to decrease beyond this point, the expression for K becomes imaginary, and putting $k' = ik$ the equation may be written

$$\left(1 - \frac{c^2}{c_0^2}\,\frac{m+1}{m}\right)\left(\frac{iJ_1(iha)}{haJ_0(iha)} + \frac{\frac{c^2}{c_0^2}\,\frac{m+1}{m} - 1}{\frac{c^2}{c_0^2}\,\frac{(m-2)(m+1)}{(m-1)\,m} - 1}\right) = \frac{iJ_1(iha)}{haJ_0(iha)}\left(1 - \frac{kaJ_0(ika)}{iJ_1(ika)}\right)$$

in which the different terms have the following signs

pos.	neg.	pos.	neg.	neg.
>0.5	<0.3	$>\frac{m-1}{m}$		

As far as the signs are concerned, the modified equation continues to furnish solutions. The left hand side is always positive and remains constantly smaller than unity, whereas the bracket on the right hand side may assume very large values for large ka. In order to satisfy the equation, the expression $\frac{iJ_1(iha)}{haJ_0(iha)}$ must become very small, or iha very large. For extremely large values, for instance, of either f or a, the two sides can become equal only when k is about equal to h, or when c equals zero.

The equation of propagation of longitudinal waves shows, therefore, that the velocity decreases steadily when the frequency, f, increases. This causes a rapid decrease of the wave-length, the rate depending, however, on the diameter of the cylinder. This change influences the rule according to which the higher harmonics would be produced in a bar of quartz and must be considered in explaining deviations from the simple spectrum of overtones that have been observed in the case of quartz crystals (3). Furthermore, because the velocity decreases to below c_0 for rapid vibrations, the fundamental frequency itself of a finite cylinder is lowered. This circumstance may cause the grains of which the metal is composed to act as resonators at much lower frequencies than would be the case for a constant velocity of propagation. In steel the length of the particle size varies from 0.01 to 1 mm., depending on the treatment, so that it does not seem impossible that the resonance frequency of the grains can be attained in ultrasonic experiments. The laws of propagation would then be completely changed, and true anomalous dispersion might be produced.

Motion of Particles

The equation giving the velocity of longitudinal waves in a cylinder involves the assumption that the motion u in the direction of the radius and w along the axis of any particle of the rod is given by the following solution of the general theory:

$$u = [-AhiJ_1(ihr)+C\gamma J_1(kr)]e^{i(\gamma z+pt)}$$
$$w = [+A\gamma iJ_0(ihr)+kCiJ_0(kr)]e^{i(\gamma z+pt)}$$

or

$$u = [-AhiJ_1(ihr)+C\gamma J_1(kr)]\cos(\gamma z+pt)$$
$$w = [-A\gamma J_0(ihr)-CkJ_0(kr)]\sin(\gamma z+pt)$$

where $\gamma=p/c=2\pi f/c$, z=distance along axis of cylinder, r=distance along radius, t=time, and A and C constants.

The constants A and C have to be chosen so as to conform with experimental conditions, for instance, certain initial or boundary requirements. The condition may be imposed, for instance, that all the points belonging to a certain end section are in phase and have the same amplitude as when they are set in motion by a rigid oscillating piston. In reality, the two components which necessarily make up such a system, cannot always be considered as separate vibrating bodies, but must be treated as a whole. However that may be, as in the equation for w the value of $-J_0(ihr)$ increases with hr whilst that of $-J_0(kr)$ decreases, the condition of w being constant over a whole cross section may be approximately fulfilled in many cases. If it is not realized, a loose contact will be produced between the source of sound and the cylinder and less energy will be transmitted. In the equation for u, if A and C are positive, both terms in the brackets increase on going from smaller to larger distances, r, a feature which might be expected.

When, on increasing the frequency, the velocity falls steadily, the moment arrives when k becomes imaginary. At this point the vibrational motion suffers a sudden change, it becomes equal to

$$u = -AhiJ_1(ihr)\cos(\gamma z+pt)+C\gamma iJ_1(ikr)\sin(\gamma z+pt)$$
$$w = -A\gamma J_0(ihr)\sin(\gamma z+pt)+CkJ_0(ikr)\cos(\gamma z+pt)$$

with $k<h$ or

$$c\,u/p = -AHiJ_1(ihr)\cos(\gamma z+pt)+CiJ_1(ikr)\sin(\gamma z+pt)$$
$$c\,w/p = -AJ_0(ihr)\sin(\gamma z+pt)+CKJ_0(ikr)\cos(\gamma z+pt)$$

The point requires further experimental study.

Radial Vibrations

Whereas at sufficiently high frequencies motions w of the particles along the axis are accompanied by strong motions u along the radius, vibrational motions u along the radius alone may exist independently of other types of vibration. The case may therefore occur where the energy of lateral vibrations is used up by radial motions of the particles. The simplest equation for purely radial displacements in a cylinder of finite length is (1)

$$u = U(r)e^{ipt}=AJ_1(gr)e^{ipt}$$

where $$g^2 = \frac{p^2}{c_0}\sqrt{\frac{(m-2)(m+1)}{(m-1)\;m}}$$

and the boundary conditions for a cylinder of finite radius a give

$$(m-1)gaJ_0(ga)-(m-2)J_1(ga)=0$$

The values of ga satisfying this equation are:

2.0694	8.5758	14.8861	21.1802
5.3958	11.7346	18.0241	24.3251

They determine the resonant frequencies, $f_1, f_2 \ldots$, for radial vibrations in a cylindrical bar:

$$f_1 = \frac{2.0694c_0}{2\pi a}\sqrt{\frac{(m-1)\ m}{(m-2)(m+1)}}\ ,$$

$$f_2 = \frac{5.3958c_0}{2\pi a}\sqrt{\frac{(m-1)\ m}{(m-2)(m+1)}}\ ,$$

and so on, for the higher modes. The overtones are evidently not harmonic, but tend toward a definite limit, forming finally a broad band of frequencies. For different values of m the first resonant frequencies are

	f_1	=	$\frac{0.34c_0}{a}$	$\frac{0.35c_0}{a}$	$\frac{0.36c_0}{a}$	$\frac{0.38c_0}{a}$	$\frac{0.48c_0}{a}$
for	m	=	8	5	4	3	2.5

that is, frequencies which are only slightly lower than those for which

$$\frac{c^2}{c_0^2} = \frac{m}{2(m+1)}$$

It is therefore probable that when a longitudinal wave belonging to this region of frequencies is sent through the cylinder, it will set up standing radial waves. The frequency at which the first double system of waves may be expected is higher the thinner the rod. The intensity of the radial motions will be stronger the higher the value of m; the two systems will necessarily react upon each other, forming a system of coupled vibrations. If we assume, as seems probable from the relations governing such a system, that the velocity of propagation of the radial waves is equal to that of the ordinary longitudinal (plus radial component) waves, then very closely

$$\text{radial wave-length} = \frac{2/3\ c_0}{1/3\ c_0}\ a$$

that is, the wave-length of the standing radial waves is determined by the diameter of the rod. In other words this dimension determines an acoustical resonance frequency of the rod, and the properties of the rod with respect to sound waves are governed by resonant frequencies as optical properties are governed by the wave-length of the resonance line. It is indeed in the neighborhood of waves of this length that anomalous dispersion of sound waves has been discovered in cylindrical rods (2).

Acknowledgment

This work was proposed by Dr. R. W. Boyle and thanks are due to him and also to Mr. G. S. Field for suggestions received.

References

1. AIREY, J. R. Arch. Math. Phys. 20: 289-294. 1913.
2. BOYLE, R. W. and FROMAN, D. Nature, 126: 602-603. 1930.
3. GIEBE, E. and SCHEIBE, A. Ann. Physik, 9: 93-175. 1931.
4. MUZZEY, D. S. Phys. Rev. 36: 935-947. 1930.
5. POCHHAMMER, L. J. Math., Crelle 81: 324-336. 1876. Summarized by A. E. H. Love, Math. Theory of Elasticity.

RESISTANCE THERMOMETERS FOR THE MEASUREMENT OF RELATIVE HUMIDITY OR SMALL TEMPERATURE DIFFERENCES[1]

By D. C. Rose[2]

Abstract

The instrument described in this paper was designed for the measurement of relative humidity in the slip stream of flying aircraft. As the temperatures to be measured are low, usually between 0° and −10° C., the temperature difference between wet and dry bulb thermometers is sufficiently small that, if mercury thermometers are used, in order to obtain the accuracy required, they would have to be of such fine bore that they could not be read from any distance. As a result wet and dry bulb resistance thermometers have been built connected to a special bridge circuit by means of which the temperature difference can be measured to 0.1° C. or better. The actual dry bulb temperature can be read by a change in the circuit or by a mercury or alcohol thermometer of fairly large bore, which can be read at some distance. In the bridge circuit the two thermometers form two arms of a bridge so that temperature variations in the leads are automatically compensated. A slide wire forms a part of the bridge circuit and the constants are so arranged that a very simple relation gives the difference in temperature of the wet and dry bulbs.

The instrument described in this paper resulted from some investigations being carried out by the author on the atmospheric conditions in the slip stream of flying aircraft. One of the measurements to be made was relative humidity. As the temperatures are rather low, often between 0 and −10° C., the temperature difference between wet and dry bulb thermometers is small enough to require accurate thermometers. If mercury or alcohol thermometers are used the scale divisions should not be more than 0.2° C. apart. Such thermometers necessarily have a small bore and cannot be read from any great distance. In the case in which the author was interested it was found impossible to read precision thermometers which had to be some distance outside the cabin of the aircraft, so it was decided to attempt to use other methods of measuring the temperatures. Methods of measuring relative humidity other than wet and dry bulb thermometers were considered but none seemed as reliable at the low temperatures involved. Hence it was decided to attempt to build satisfactory wet and dry resistance thermometers.

The quantity which must be measured most accurately is the difference between the wet and dry bulb temperatures rather than the actual temperatures. The actual dry bulb temperature can be measured with a thermometer of fairly large bore which can be read from a distance. With this in view the resistance thermometers described here were connected as two arms of a Wheatstone bridge using a 1:1 ratio. The arrangement does not give the temperature of either thermometer but with a very simple type of slide wire forming the other arms of the bridge, the temperature difference between the wet and dry thermometers could be read easily to 0.1 °C. and at the same time the whole apparatus, including the batteries, galvanometer and

[1] *Manuscript received June 6, 1931.*
Contribution from the National Research Laboratories, Ottawa. This paper was read before Section III of the Royal Society of Canada in May, 1931.
[2] *Assistant Research Physicist, National Research Laboratories, Ottawa.*

slide wire, was sufficiently rugged to withstand the vibration in an aeroplane and did not weigh more than a few pounds. Further the apparatus automatically compensates itself for temperature changes in the leads.

The circuit used is shown in Fig. 1. W and D are the wet and dry resistance thermometers (approximately 100 ohms each). It will be seen that the calculation of the results is simpler if the resistances of these two thermometers are the same, but the range of the instrument is increased if they are slightly different. In the apparatus built by the author they happened to be the same, more by accident than by intention. The resistances A and B and the slide wire were simply a Leeds and Northrup "Students Kohlrausch Slide Wire"*.

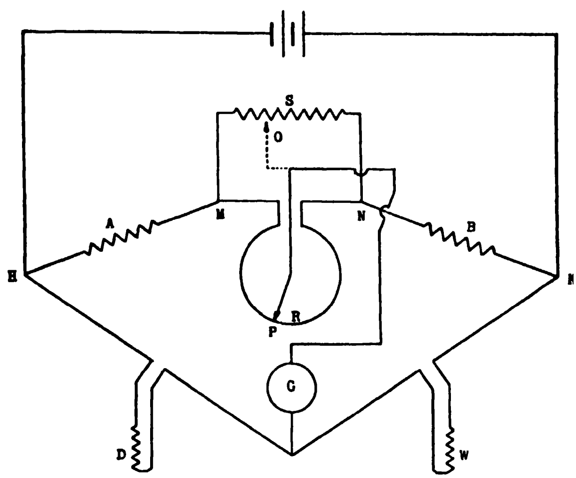


FIG. 1. *Bridge circuit used with resistance thermometers.*

The slide wire has a resistance of 121.4 ohms and the scale reads from 0 to 1000, every five divisions being marked. The resistances of A and B are respectively 546.3 and 546.2 ohms. The purpose of the resistance S is to change the sensitivity of the slide wire. The resistance of the slide wire without S formed too large a portion of the bridge circuit. By varying S a change of 2° C. in the temperature of one of the thermometers could be made to correspond to any number of divisions from a few to 500 on the slide wire.

The process of measuring the temperature differences between D and W is very simple. First the position of the slide for a balance when D and W are at the same temperature must be known. This can be found easily by putting them both in the same temperature bath. If D and W have the same resistance, this position is practically at the centre of the slide wire, as A and B have practically the same resistance. This position will also be independent of S. If D and W have not the same resistance the position of the balance with D and W at the same temperature will depend on S and must be known for whatever value of S is used. The position of this balance is always the same and in the apparatus used by the author remained the same when the whole was dismantled and connected up in the aeroplane. Then with W and D as a wet and dry bulb hygrometer, a balance can be found and the difference in temperature between W and D can be calculated from the equations given below.

Analysis of the Circuit

The circuit shown in Fig. 1 may be treated as a simple bridge circuit by considering a point O on the shunt S at the same potential as the point P on

*Catalogue No. 4261.

the slide wire. When the bridge is balanced such a point always exists. The introduction of the shunt S in the bridge circuit now means that in place of MP in the simple bridge circuit, the effective resistance of MO and MP in parallel must be used. Hence the resistance of the arm HP is $A+\frac{MP.\,MO}{MP+MO}$ and similarly of the arm KP it is $B+\frac{NP.NO}{NP+NO}$. The conditions for balance are now

$$\frac{D}{W}=\frac{A+\dfrac{MP.\,MO}{MP+MO}}{B+\dfrac{NP.\,NO}{NP+NO}} \tag{1}$$

put this $=\frac{x}{y}$. The quantities observed on the slide wire are MP and PN. The slide wire scale is numbered from 0 to 1000 so that if the reading be divided by 1000, a fraction, θ, of the total resistance of the slide wire is obtained. The resistance $MP=\theta R$ and $PN=(1-\theta)R$. The equation (1) may be reduced to

$$\frac{D}{W}=\frac{x}{y}=\frac{A+C\theta R}{B+C(1-\theta)R} \tag{2}$$

where $C=\frac{S}{R+S}$, R and S being the resistances of the slide wire and shunt respectively.

The resistances of the thermometers D and W are to a first approximation $D=D_0(1+\alpha t_D)$ and $W=W_0(1+\alpha t_W)$, where D_0 and W_0 are the resistances of the thermometers at some specified temperature in the range of temperatures being measured; α is the temperature coefficient of the resistance of the wire in the thermometers, and t_D and t_W are the differences between the specified temperature and the temperatures observed. The equation may be written

$$\frac{x-y}{y}=\frac{D-W}{W}=\frac{D_0(1+\alpha t_D)-W_0(1+\alpha t_W)}{W_0(1+\alpha t_W)}$$

Assume for the present that $D_0=W_0$, then

$$\frac{x-y}{y}=\frac{\alpha(t_D-t_W)}{1+\alpha t_W}.$$

In practice the quantity αt_W must always be small compared with unity: hence it may be omitted in the denominator and the equation may be written

$$\frac{x-y}{y}=\alpha\,(t_D-t_W)$$

and from (2)

$$=\frac{A+C\theta R-\{B+CR(1-\theta)\}}{B+CR(1-\theta)} \tag{3}$$

and the difference in temperature of the two thermometers may be calculated easily from the known constants of the circuit.

A further approximation which simplifies the calculation may be made. A and B are effectively equal so may be disregarded in the numerator. C is in practice considerably less than 1 otherwise the shunt S is useless. $(1-\theta)$ is considerably less than 1 particularly at the top of the scale. R in the apparatus used was $\frac{2}{9}B$; C was usually between $\frac{1}{6}$ and $\frac{1}{20}$ in all the tests made and if $W_0=D_0$, then $(1-\theta)$ is never more than $\frac{1}{2}$. Hence the term $RC(1-\theta)$ is

never greater than $\frac{2}{9}\cdot\frac{1}{6}\cdot\frac{1}{2}B=\frac{1}{54}B$. Therefore if we neglect this term in the denominator the error involved is less than 2%. Where differences of the order of 2° C. are to be measured, this error is less than possible errors in reading two mercury thermometers even if fairly high precision thermometers are used. The equation now becomes

$$t_D - t_W = \frac{RC(2\theta-1)}{\alpha B}. \tag{4}$$

The constant $\frac{RC}{\alpha B}$ may be evaluated for the different values of S used.

The resistance thermometers were made of nickel wire 0.15 mm. in diameter and wound on a hollow ebonite spool. As the wire was bare, a thread (96 to the inch) was turned on the spool to keep it in place. The spool was about 1¼ in. long and ½ in. in diameter. It was hermetically sealed in a glass tube filled with a good grade of oil. The oil was added as an insulator and to improve the thermal conductivity between the glass and the wire. This construction of course caused some lag in the resistance wire taking up the temperature of the glass tube, but trials showed that readings could be taken after temperature conditions had been steady for six or seven minutes. The resistance of the thermometers was about 97 ohms and the temperature coefficient of resistance was found to be 0.00284 ohms per ohm per degree between 0 and 20° C.

Actually no attempt was made to make the two resistances exactly the same and when they were sealed up one was slightly higher than the other, but after seasoning they came by accident to the same value so that the balance, when they were at the same temperature, was exactly in the middle of the slide wire. An examination of the above theory shows that there is nothing gained by making them the same If they are different the same formula applies but an additional calculation must be made. The thermometer having the higher resistance should be the wet one and the balance found when the two are at the same temperature, then as the wet thermometer becomes cooler than the dry, the position of balance will move towards the centre of the slide wire scale. A calculation must be made for the difference in temperature required to bring the position of balance to the centre of the scale. This must be added to the difference found when the final reading is taken. There is in fact a slight advantage in having the resistance of the two thermometers slightly different as a greater part of the slide wire scale is usable.

The most suitable value of S depends on the temperature difference to be measured. For differences up to 10° C. a convenient value was found to be about 25 ohms. If S was made smaller than about 5 ohms the position of balance could not be found so accurately, since the galvanometer current was so much reduced. A small resistance box was made for S, having four coils with resistances of 4.38, 6.17, 8.00 and 10.54 ohms. These could be used either individually or in series. The galvanometer was a portable Leeds and Northrup insulation testing set consisting of a portable galvanometer arranged with a telescope and scale and containing a universal shunt, the whole being very conveniently mounted. This was found to be rugged enough for use in

aircraft though if there was excessive vibration the instrument had to be held on the author's knee in order to obtain a good balance. This was not found inconvenient.

In order to check the above theory, readings were taken with the two thermometers in different temperature baths. The following is a set of results:

Balance with both at the same temperature, 500

Balance with one at 17° C. and the other at 7.7° C., 920

Resistance of shunt, S, 20 ohms

$\alpha = 0.00284$ ohms per ohm per °C.

According to formula (4)

$$t_D - t_W = \frac{121.4 \times 0.141 \times 0.840}{0.00284 \times 546.2} = 9.26$$

and as $17.0 - 7.7 = 9.3$, it is seen that the agreement is very good, and one-tenth of a degree was the greatest accuracy hoped for, with the equipment used. Using formula (3) the result would have been about $\frac{1}{3}$% higher making the agreement even better.

A large number of humidity tests were made to try out the instrument in the laboratory. Some of the readings are recorded below. The results are compared with those obtained from wet and dry bulb mercury thermometers placed beside the resistance thermometers both being ventilated by an electric fan.

TABLE I

COMPARISON OF RESISTANCE THERMOMETERS WITH MERCURY THERMOMETERS

Wet bulb temp., °C.	Dry bulb temp., °C.	Balance of bridge	Resistance of S, ohms	$t_D - t_W$ (temp. difference)	
				Formula (4) °C.	Mercury thermometers °C.
10.6	20.3	866	25.00	9.8	9.7
9.3	19.1	835	29.07	10.2	9.8
9.7	19.6	945	18.53	9.2	9.9
11.6	21.0	965	18.53	9.6	9.4
8.9	15.4	814	18.53	6.5	6.5
11.5	20.8	956	18.53	9.4	9.3

In the case of the first three of the above, the room in which the readings were taken was well ventilated by open windows. In the case of the latter three, the windows and doors were closed in order to obtain constant humidity. In view of the difficulty in obtaining constant humidity in a large room containing two sinks as well as a considerable quantity of apparatus the results show very good agreement. Even the proximity of the observer produces a detectable change in the humidity and, as the time lag of the mercury thermometer and the resistance thermometers was very different, great precision could not be obtained unless very special precautions were taken. This time lag in the resistance thermometers seemed unavoidable, as any design of the thermometers which would allow one of them to be kept wet in the usual manner would require the elements well insulated from the water.

Measurements of relative humidity taken in the slip stream of flying aircraft will no doubt be published later. So far the instrument has been tried out on two flights and found to be practical. Its use however is not limited to relative humidity measurements. Wherever temperature differences are required it can be used and by a change-over switch and an additional fixed resistance the temperature of either thermometer could be measured by making the apparatus into an ordinary bridge. If the instrument were to be used in a laboratory the accuracy could be improved a great deal by using precision resistance boxes and a very sensitive galvanometer. It should be easy to measure temperature differences varying from a few degrees to 0.01° C.

It is unlikely that this bridge circuit has never been used before, or that resistance thermometers have never been used for relative humidity measurements but, as far as the author is aware, this combination is new and seems especially well adapted for measurements where small temperature differences are involved.

AN INVESTIGATION OF THE EQUILIBRIA EXISTING IN GAS-WATER SYSTEMS FORMING ELECTROLYTES[1]

By O. M. Morgan[2] and O. Maass[3]

Abstract

The data and theoretical treatment contained in this paper are the continuation of a series of researches instituted to investigate the equilibria existing in certain gaseous-aqueous systems. In this work the vapor pressures and electrolytic conductivities of aqueous solutions of sulphur dioxide, carbon dioxide, and ammonia have been measured with greater precision than ever before over a temperature range from 0 to 25° C. and over a concentration range where their respective vapor pressures do not exceed one atmosphere. From the data thus derived, equilibria relations have been calculated and certain changes have been made in the mode of theoretical procedure involved in this type of calculation.

With regard to the equilibria existing in the three systems, the conclusions drawn may be summarized as follows: Practically all of the ammonia exists in the combined form and practically all the carbon dioxide exists as free carbon dioxide in the aqueous solutions in the temperature range investigated. The amounts of free and combined sulphur dioxide are of the same order of magnitude and the relative amount of combined and free sulphur dioxide can be calculated approximately, the latter increasing markedly with rising temperature. The true dissociation constant can therefore be found for ammonium hydroxide, can be estimated for sulphurous acid, but cannot be calculated for carbonic acid from the available data.

Introduction

Sulphur dioxide, carbon dioxide, and ammonia are very common materials and are widely used in many industries. It is amazing, when a search is made in the literature, to find that the data which are of such economic interest are either lacking or inaccurate. Perhaps the sulphur dioxide-water system has been investigated more thoroughly than the other two.

The solubility of sulphur dioxide in water was determined by Hudson (19) working at approximately atmospheric pressure and temperatures up to 90° C. Sims (44), working at pressures up to 200 cm. and temperatures up to 50° C., measured the vapor pressures with considerable accuracy. Enckell (11) and Oman (39) measured solubilities up to 90° C., Enckell using less exact methods than Oman. Enckell also measured the solubility of sulphur dioxide in other solutions such as calcium bisulphite, sodium sulphate plus hydrochloric acid, and sulphuric acid. Vapor pressure measurements of solutions of sulphur dioxide in water, and in water plus various amounts of bases were made by Smith and Parkhurst (45).

Ostwald (40) and Barth (2) carried out investigations on the conductivity of sulphur dioxide solutions. McRae and Wilson (33) determined partition coefficients of sulphur dioxide between water and chloroform. Walden and Centnerszwer (47) made freezing point determinations. Drucker (10, p. 579) combined the results of the four previous investigations to calculate the so-

[1] *Manuscript received June 9, 1931.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada.

[2] *Assistant Research Chemist, National Research Laboratories, Ottawa, and holder, at the time, of a studentship and fellowship under the National Research Council of Canada.*

[3] *Professor of Physical Chemistry, McGill University.*

called dissociation constant. Kerp and Bauer (23) measured freezing points and conductivities. Fulda (17) gives figures for the dissociation constant at temperatures from 2 to 50° C. These he has calculated from the heat of dissociation data of Thomsen (46) and Berthelot (4). Lindner (28) reviews and discusses these papers and has also made determinations of vapor pressure at 0, 25 and 50° C. on four solutions ranging in concentration from 0.05 to 3.8%. He also made conductivity measurements of the following solutions:—6.20% at temperatures from 0 to 39.9° C.; 3.24% at temperatures from 12.7 to 56.7° C.; 0.34% at temperatures from 9.5 to 69.9° C.; 0.05% at temperatures from 0 to 70.5° C.

Any other available data on sulphur dioxide have been obtained in this laboratory. C. Maass (31) made vapor pressure and conductivity measurements over all possible concentrations below 27° C.

The range of most interest to those concerned with the sulphite process of cooking wood pulp is that between 0 and 6% concentration and at 100° C. or slightly above. This range has been covered by W. B. Campbell and O. Maass (8) in this laboratory. The apparatus used by these investigators was designed to measure pressures up to four atmospheres and hence did not give precise measurements at the lower pressures. Their reaction chamber was small, which also tended to cut down the precision, and the maximum concentration attained was only 8%. In order to obtain a true measure of the equilibria, as will be seen later, it is necessary to have a comparatively large variation in the water concentration. In the present work concentrations as high as 14% sulphur dioxide were attained which gave approximately twice the variation in the water concentration that was reached by Campbell. Campbell and Maass admit that the values of their equilibria constants are only approximate. The aim of this work was to arrive at a true evaluation of these constants and to investigate the type of equilibria existing at the lower temperatures.

Considerable data are available for carbon dioxide. Bohr (5) working over a temperature range of 0 to 60° C. and over a vapor pressure range of 27 to 140 cm. has determined the Henry's Law constants. Findlay and Creighton (12) measured the solubility of carbon dioxide in water and the effect of fine suspensions on the solubility. Findlay and Williams (15) determined the solubility of carbon dioxide in water at pressures lower than one atmosphere. Findlay and Shen (14) investigated the effect of colloids and fine suspensions on the solubility of carbon dioxide in water. The results were in harmony with Henry's Law. Findlay and Howell (13) measured the vapor pressures of carbon dioxide in pure water and in starch solutions at 25° C. for pressures from 272 to 960 mm. Buch (6) determined the solubility of carbon dioxide in water at temperatures between 17 and 20° C. and calculated the absorption coefficients. Just (20) working at 25° C. determined Henry's Law constant and obtained a value which agreed well with that of Bohr (5) but differed from the rest.

Conductivity data for carbon dioxide are not quite so plentiful. Those given by Knox (24), Pfeiffer (42) and Walker and Cormack (49) are reviewed in a concise manner by Kendall (22) who also supplies considerable data. The

above experimenters worked at temperatures from 0 to 25° C. and concentrations up to 0.08 gm.-mols per litre. The data of Kendall (22) agree very well with those due to Walker and Cormack (49) but do not compare favorably with those of Pfeiffer (42) or Knox (24). Wilke (50) attempts to explain the equilibria of carbon dioxide in water solutions. He determined the dissociation constants by conductivity measurements at various temperatures and showed carbonic acid to be a very weak acid. This acid is shown to be a sensitive compound and is decomposed by slight influences such as the current used for measurement.

Vapor pressures of solutions of ammonia at 0, 20 and 40° C. and up to 3640 mm. have been measured by Neuhausen and Patrick (35). Foote (16), working at 10 and 20° C., determined the vapor pressures up to 1500 mm. As a side issue they investigated the equilibrium in the system ammonia-ammonium nitrate-ammonium thiocyanate with the object of discovering what proportions of these substances were most efficient in absorbing ammonia from gas mixtures. Perman (41, p. 1397) has also covered the lower range of vapor pressures.

Kanolt (21) determined the ionization constant of ammonium hydroxide at 0, 18 and 25° C. Lunden (29, 30) has measured the affinity coefficients of ammonia solutions between 15 and 40° C. and conductivities between 10 and 50° C. in dilute solutions. Noyes and Kato (38) measured the conductivity and determined the ionization up to 156° C. using a steel bomb lined with platinum electrodes which were insulated away from the walls. Burke (7) investigated the ionization of aqueous solutions of ammonia in the presence of urea to find if an ammonia-urea complex were formed. The results showed that such appeared to be the case. Specific conductivity data are also provided by Kohlrausch (25, p. 1078).

The density of ammonia solutions up to 40% concentration and between −15 and 25° C. have been determined by Nichols and Wheeler (36, p. 59) and Baud and Gay (3).

A general summary of the work carried out by other investigators on all three systems shows considerable lack of agreement apart from the total lack of data in certain concentration and temperature regions. One special source of controversy is to be found, not in the accuracy of actual pressure or conductivity measurements, but rather in the determination of the concentration factor. Special emphasis is laid, in the experimental work to be described, on the elimination of all doubt as to the true concentrations, and of true equilibria having been reached between the gaseous and liquid phase. This may have been another source of discrepancies obtained by other investigators.

It would lead too far afield to give a detailed account of all theories that have been put forward in connection with the equilibria existing in the various systems investigated. Due to the fact that the work described in this paper is a continuation of the work begun by C. Maass (31) and Campbell and Maass (8), as far as sulphur dioxide is concerned, it is convenient to overlap their work. On the experimental side this will be done by the comparison of refinements and improvements that have been made. The need for the

extreme precision is brought out by the following review of the theoretical considerations as they have been developed so far. It will be seen that previous data are not adequate in testing it out.

In this theoretical discussion terms peculiar to solutions of sulphur dioxide will be used. This discussion will contain the major number of steps necessary in dealing with gaseous-aqueous equilibria and all the steps necessary for sulphur dioxide as evolved by the above experimenters. However, certain characteristics, depending on the gas under consideration, necessitate minor changes in the mathematical treatment. These peculiarities will be dealt with in discussing the respective systems in later sections.

In aqueous solutions of sulphur dioxide there are three major and one minor equilibria existing. The partial pressure of the sulphur dioxide above the solution is in equilibrium with the uncombined gas in the solution.

$$[SO_2]_{solution} = H[SO_2]_{gas} \tag{1}$$

and since the partial pressure of the gas above the solution is a measure of the concentration of the gas in the vapor

$$[SO_2]_{solution} = Hp \tag{2}$$

where p is the partial pressure of the gaseous sulphur dioxide and H is a constant (Henry's constant).

The sulphur dioxide molecules in the solution are in equilibrium with sulphurous acid and may be represented

$$H_2O + SO_2 \rightleftarrows H_2SO_3 \tag{3}$$

and, applying the mass law,

$$[H_2O]\ [SO_2] = K_1\ [H_2SO_3] \tag{4}$$

The sulphurous acid in solution is ionized to a certain extent and is in equilibrium with its ions

$$H^+ + HSO_3^- \rightleftarrows H_2SO_3 \tag{5}$$

and assuming that the Ostwald dilution law holds in this case,

$$[H^+]\ [HSO_3^-] = K_2\ [H_2SO_3] \tag{6}$$

A further ionization is possible according to the equation

$$H^+ + HSO_3^- \rightleftarrows 2H^+ + SO_3^= \tag{7}$$

but this ionization is so small it may be safely neglected.

The problem now presented is the evaluation of the constants H, K_1, and K_2. Previous investigators have merely calculated the apparent dissociation constant obtained by the assumption that all the sulphur dioxide in the solution is combined with water to form H_2SO_3. That is

$$\begin{aligned}[H^+]\ [HSO_3^-] &= K_a\ ([H_2SO_3] + [SO_2]) \\ &= K_a \left(\frac{[SO_2\ ]\,[H_2O]}{K_1} + [SO_2]\right) \\ &= K_a\,[SO_2] \left(\frac{[H_2O]}{K_1} + 1\right) \end{aligned} \tag{8}$$

then since

$$[H^+][HSO_3^-] = K_2[H_2SO_3] = K_2\frac{[SO_2][H_2O]}{K_1}$$

the relation between K_1, K_2, and K_a is

$$K_a = \frac{K_2[H_2O]}{K_1+[H_2O]} \tag{9}$$

The first attempt at the actual evaluation of the constants was made by C. Maass (31) as follows:—

$$C_{H_2O} = [H_2O] + [H_2SO_3] + [HSO_3^-] \tag{10}$$

$$C_{SO_2} = [SO_2] + [H_2SO_3] + [HSO_3^-]. \tag{11}$$

On subtracting (10) from (11)

$$[H_2O] = C_{H_2O} - C_{SO_2} + [SO_2] = C_{H_2O} - C_{SO_2} + Hp \tag{12}$$

$$C_{SO_2} = [SO_2] + \frac{[SO_2][H_2O]}{K_1} + \sqrt{K_a[SO_2]\frac{[H_2O]}{K_1} + 1} = Hp\left(1+\frac{[H_2O]}{K_1}\right) + \sqrt{K_a\, Hp\left(1+\frac{[H_2O]}{K_1}\right)},$$

and substituting (12)

$$C_{SO_2} = Hp\left(1+\frac{C_{H_2O} - C_{SO_2} + Hp}{K_1}\right) + \sqrt{K_a Hp\left(1+\frac{C_{H_2O} - C_{SO_2} + Hp}{K_1}\right)}. \tag{13}$$

This equation contains two unknowns, H and K_1. The value of K_a was obtained from conductivity measurements. By taking two values of C_{SO_2} at any one temperature and the corresponding pressures it is possible to arrive at the value of H and K_1, and hence K_2, the true dissociation constant. It must be remembered at this point, however, that to get an accurate evaluation of the constants there must be considerable variation in the water concentration, as was mentioned previously. Vapor pressures must also be measured very accurately. For the above reasons C. Maass (31) was able to arrive at only approximate values of the constants. It was found from the calculations that at 15° C. the amount of uncombined sulphur dioxide in the solution amounted to about 20% of the total amount dissolved, whereas at 23° C. the amount of uncombined sulphur dioxide was about 50% of the total, that is, K_1 increased rapidly with temperature. This was for a solution of 5% concentration in each case. The value of the true dissociation constant was found to be approximately 0.02 and did not change much with temperature. It will be shown later from this work that all previous data below 12° C. have been at fault.

Campbell and Maass (8) made a somewhat different use of their data. Sulphurous acid may be assumed to behave as a strong acid, which is reasonable because the organic sulphonic acids as a group are strong acids. They assume

that sulphurous acid is almost completely ionized. Then, instead of considering that water and sulphur dioxide combine to form sulphurous acid which in turn dissociates into its ions, the mechanism may be considered analogous to the formation of NH_4^+ from NH_3+H^+ and the reaction written thus:

$$SO_2 + OH^- + H^+ \rightleftharpoons HSO_3^- + H^+.$$

The extent of the reaction will be determined by the equilibrium expressed as follows:

$$[SO_2]\,[OH^-] = K_3[HSO_3^-]$$

and

$$[H^+]\,[OH^-] = K_w[H_2O]\,.$$

Combining these

$$[SO_2]\,[OH^-] = \frac{[SO_2]K_w\,[H_2O]}{[H^+]} = K_3[HSO_3^-]$$

or

$$H^+[HSO_3^-] = \frac{K_w}{K_3}[SO_2]\,[H_2O]$$

$$= K_b(C_{SO_2} - [HSO_3^-])\,[H_2O] \qquad (14)$$

also

$$[SO_2] = C_{SO_2} - [HSO_3^-] = Hp \qquad (15)$$

Equation (15) may be derived from equation (13) as follows: Equation (13) may be written

$$C_{SO_2} = Hp\left(1 + \frac{[H_2O]}{K_1}\right) + [HSO_3^-] \qquad (13a)$$

If in the first place K_1 is large, or secondly $[H_2O]$ considered to be constant, equation (13) then becomes

$$C_{SO_2} = Hp + [HSO_3^-]$$

or

$$C_{SO_2} - [HSO_3^-] = Hp$$

At high temperatures the first is true and at low concentrations the second is true, so that the data obtained by Campbell and Maass (8) are not adequate to distinguish between equations (13) and (15).

When the data of Campbell and Maass were used and p, the partial pressure of sulphur dioxide, was plotted against the values of $C_{SO_2} - [HSO_3^-]$ a straight line was obtained as was required by equation (15). It must not be concluded though that no sulphurous acid exists as such since the range of dilution in their work at high temperatures is not great enough to affect the proportionality of $[H_2SO_3]$ to $[SO_2]$ when $[H_2O]$ is constant. At any rate it would be too small to be evident in the plot. In this case the two constants instead of three are used to express the equilibria, *i.e.*, H and K_b. K_b, obtained in equation (14), bears a relation to the apparent dissociation constant through the following equations:

$$[H^+]\,[HSO_3^-] = K_a(C_{SO_2} - [HSO_3^-])$$

$$= K_b(C_{SO_2} - [HSO_3^-])\,[H_2O]\,.$$

Hence

$$K_a = K_b\,[H_2O]\,.$$

It may be emphasized again that the measurements at high temperatures will be in accord with equation (15) because the equilibrium given by equation (4) is shifted to the left by rise in temperature as indicated by the data of C. Maass (31). Wright (51) and Baly and Bailey (1) showed by means of absorption spectra that free sulphur dioxide exists in the solution which is in agreement with this idea.

From the above summary of previous work it is seen that measurements of greatest possible precision carried out at temperatures below 25° C. are essential in the determination of the true equilibria. This requires greater refinement of measurement than was used by C. Maass (31) at low concentrations, and over the temperature range not covered by Campbell and Maass (8).

There is another reason for carrying out the work to be described apart from the use in the theory. All three systems investigated are of a particular interest from a commercial point of view. The importance of sulphur dioxide is emphasized in the sulphite cooking of wood pulp, in electrical refrigeration, as a disinfectant and preservative. Carbon dioxide is of importance to the manufacturers of carbonated beverages, while ammonia has numerous uses, the main one at the present time being that of a refrigerant.

The following section will contain a complete discussion of the apparatus and the experimental work with the exception of the respective purifications of each gas studied. Three sections will then be devoted to the presentation of the experimental results obtained for sulphur dioxide, carbon dioxide and ammonia respectively, as well as the theoretical treatment, the corresponding theoretical data, and discussions of the intrinsic value of the data.

Experimental

Apparatus

The apparatus used in the preparation of gaseous-aqueous solutions is illustrated in Fig. 1. The system may be evacuated either by a Hyvac, a water pump, or a Langmuir diffusion pump backed by a Hyvac. C is a metal cylinder containing the gas to be used and F_1, F_2, F_3, and F_4 constitute a fractionating apparatus for the purification of the gas. The pure gas is held in the liquid or solid form, as the case may be, in F_4 by packing it in a Dewar flask containing a dry ice-ether mixture. X is a rough manometer which acts as a safety valve during the successive distillations and also gives a measure of the vapor pressure of the gas which is a check on the rate of distillation.

M_1 is an all glass manometer. The left side is evacuated and the right side is connected to the volume measuring system. It is mounted with an etched mirror scale and by applying pressure or vacuum over the reservoir the mercury is always drawn to the level L_1 for reading. G is a McLeod gauge for the determination of low pressures especially when the manometers are being evacuated. V_1 and V_2 are volumes which have been calibrated carefully to 0.1 cc. The volume of the connecting tubing bounded by stopcocks T_8, T_9, T_{19}, and the level L_1 on manometer M_1 was also calibrated to 0.1 cc. From these calibrations, $V_1 = 587.8$ cc., $V_2 = 175.7$ cc., and V_4 (connecting tubing) $= 90.6$ cc.

V_3 is a small condensation bulb in which the gas was condensed before injection into the conductivity cell. E is a special type of conductivity cell constructed entirely of Pyrex glass. Stirring was effected by the electromagnetic stirrer D operated by the two solenoids B_1 and B_2, the circuit through the former being alternately made and broken by the automatic mercury valve Q, and the latter being in circuit permanently. By varying the current through the solenoids by means of the banks of lamps L_3 and L_4, any length of stroke could be obtained.

Within the cell O_1 and O_2 were platinized platinum electrodes sealed into the Pyrex by the same method as that used by Campbell and Maass (8) and originated by Housekeeper (18). The electrodes were connected to the Wheatstone Bridge through the mercury well and the leads W_1 and W_2. The side arm H

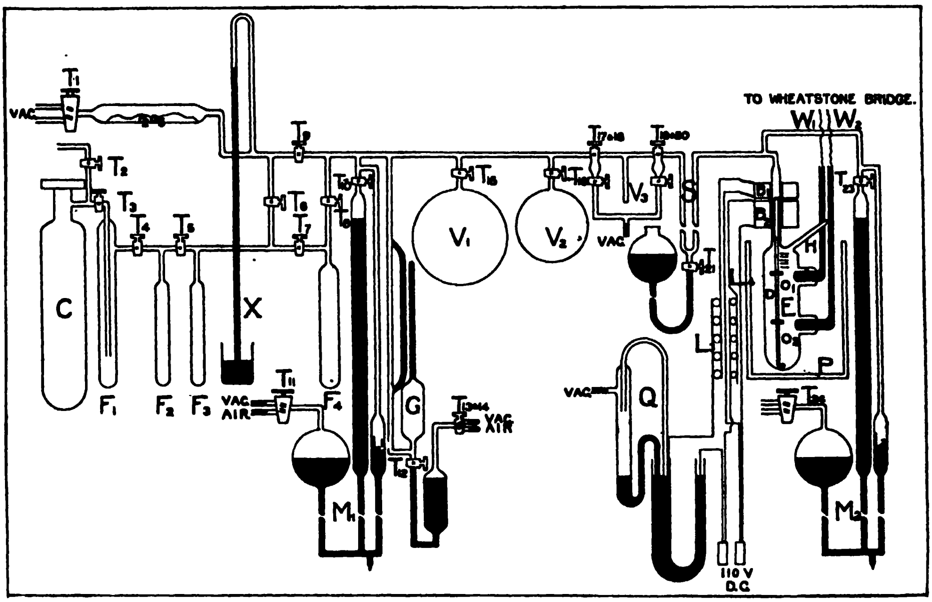


FIG. 1. *Diagram of apparatus.*

was connected to a distillation cell from which the conductivity water was distilled into the cell after a series of freezings and evacuations to rid it of any residual air. The mercury seal S provided a system free from stopcocks during the distillation.

M_2 was a glass manometer similar to M_1 and was used for the measurement of vapor pressures. At low pressures, where the concentration coefficient of the vapor pressure was small, a cathetometer was used for making the readings.

The Wheatstone Bridge consisted of a Leeds and Northrup Kohlrausch slide wire, two banks of Curtiss-wound resistance coils, one reading in steps of 1 ohm from 1 to 1000 ohms, and the other in banks of thousands reading up to 40000 ohms. These resistances were checked against standard coils and were found to be accurate. The slide wire was also calibrated and gave

satisfactory results. A condenser with a maximum capacity of 0.00012 microfarad was shunted across the resistance terminals in order to balance out any capacity that might exist in the cell at low concentrations. This aided in giving better end-points. A Vreeland oscillator, operating on a frequency of 1000 cycles, was used as a source of current. This oscillator gives a pure sine wave with no harmonics. One end of the slide wire was grounded to the plumbing in the laboratory and at no time were any body capacity effects noted. A two stage radio amplifier was used in the phone circuit; this gave much more sensitive end-points and increased the accuracy of the conductivity measurements.

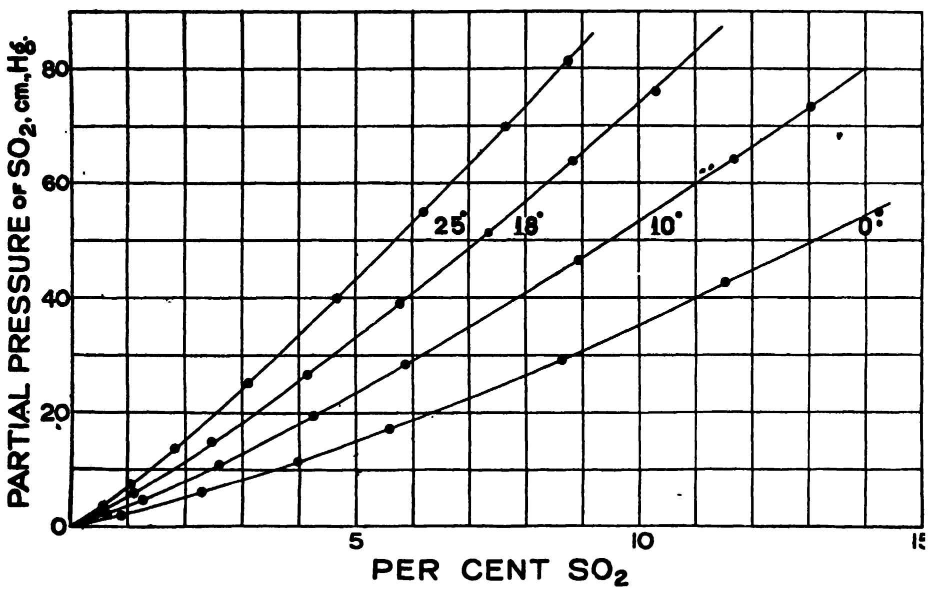


FIG. 2. *Partial vapor pressures of sulphur dioxide solutions.*

The preparation of the solutions and the calculation of the amount of gas in the solution was carried out by the method of Maass and Maass (32).

Sulphur Dioxide

As was indicated in the introduction very accurate data have to be obtained for sulphur dioxide in order to obtain a true insight into the equilibria. For this reason particular care was taken and more time was spent on this system than on the other two.

A cylinder of liquid sulphur dioxide was obtained from the Ansul Chemical Co., in Marinette, Wisconsin. Analysis showed this gas to be very pure but in order to insure the greatest purity possible it was subjected to three consecutive distillations as described in the experimental section. This gave a clear sample of liquid sulphur dioxide. Freezing point and vapor pressure determinations showed it to be extremely pure and it was considered ready for use.

The method of preparing the solutions has been described in the experimental section. In each case the temperature of the conductivity cell was held constant and the concentration was raised by consecutive injections of gas. This was considered to be a more accurate procedure than varying the temperature at one concentration. Time was also saved due to the fact that relatively high concentrations were reached and the greatest time lag was involved in the injection of the gas.

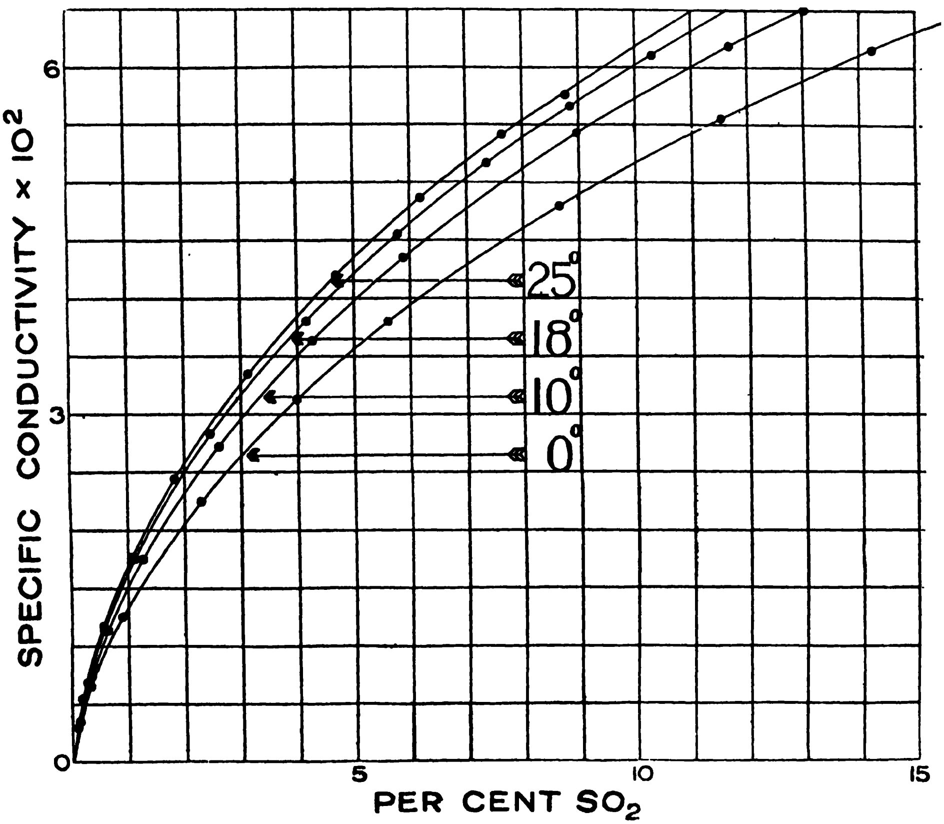


FIG. 3. *Specific conductivity of sulphur dioxide solutions.*

Results

Table I and Fig. 2 and 3 give the experimental data obtained for sulphur dioxide. It should be mentioned here that this is the second set of data for sulphur dioxide that has been obtained by the writer. A set of data was obtained in 1928-29 which was checked by a similar set in 1929-30. The two sets of results were quite gratifying inasmuch as they gave excellent checks. Only slight changes were noted in the 1929-30 conductivity measurements. These latter measurements, however, were taken as being the most nearly correct since a better conductivity apparatus, the one described in the previous section, had been used. There was no noticeable difference in the vapor pressure data.

An inspection of the conductivity and vapor pressure curves shows that they are quite regular within themselves. This is a strong argument in their favor. It was possible to cross check them by taking a certain concentration at say 10° C. and raising the temperature to 18 and 25° C. and taking vapor pressure and conductivity readings. The greatest deviation found by this method of checking was 0.1%. In this way the regularity and accuracy of procedure was proved.

TABLE I

EXPERIMENTAL DATA OBTAINED FOR THE SYSTEM SULPHUR-DIOXIDE WATER

Reading	SO_2 %	Partial pressure of SO_2, cm. Hg.	Specific conductivity	Reading	SO_2 %	Partial pressure of SO_2, cm. Hg.	Specific conductivity
At 0° C. Weight of water used = 116.010 gm.							
1	0.1369	0.17	0.00339	6	5.597	17.30	*0.03800
2	0.3374	0.63	0.00650	7	8.630	29.21	0.04804
3	0.8888	1.97	0.01253	8	11.52	41.63	0.05560
4	2.284	6.05	0.02255	9	14.23	55.02	0.06158
5	3.978	11.55	0.03135				
At 10° C. Weight of water used = 115.187 gm.							
1	0.1267	0.35	0.00366	6	4.256	19.52	0.03638
2	0.3414	1.00	0.00748	7	5.872	28.37	0.04355
3	0.6360	2.12	0.01136	8	8.937	46.62	0.05431
4	1.261	4.71	0.01759	9	11.68	64.18	0.06189
5	2.593	10.98	0.02727	10	13.03	73.58	0.06491
At 18° C. Weight of water used = 116.518 gm.							
1	0.0816	0.32	0.00296	6	4.150	26.66	0.03800
2	0.2605	1.10	0.00689	7	5.773	39.01	0.04556
3	0.5563	2.68	0.01143	8	7.340	51.44	0.05169
4	1.103	5.92	0.01757	9	8.831	63.69	0.05667
5	2.458	14.83	0.02832	10	10.30	76.04	0.06108
At 25° C. Weight of water used = 116.416 gm.							
1	0.1735	0.79	0.00545	6	4.672	40.00	0.04201
2	0.5448	3.42	0.01174	7	6.184	55.00	0.04866
3	1.056	7.37	0.01775	8	7.640	69.88	0.05416
4	1.809	13.61	0.02443	9	8.756	81.17	0.05769
5	3.116	25.28	0.03355				

At 25° C. the vapor pressure values obtained by C. Maass (31), and Campbell and Maass (8) are in agreement with themselves but are 2% higher than the values obtained in the present work. There is also an irregularity in the lower pressures amounting to about 1%. At 10° C. the results of C. Maass (31) are even more seriously in error. The apparatus used by these experimenters, as mentioned before, was designed to measure high pressures and for this reason low pressures could not be obtained with as great accuracy.

The conductivity data of C. Maass (31), and Campbell and Maass (8) check to an approximation. However, their results are 0.66% higher than the present values at the higher concentrations and increase in error at the lower concentrations. The deviation may be attributed to the use of a small reaction chamber (20-25 cc), thereby cutting down the accuracy of the determination of the concentration. Higher vapor pressures and conductivities would also point to the presence of some volatile soluble impurity. This latter is not highly probable.

The theoretical treatment of sulphur dioxide as developed to date has been discussed in the introduction. It was quite apparent that over the temperature and concentration range covered in this work that equation (13), as developed by C. Maass (31), was the most rigorous and should be the logical starting point for any further development.

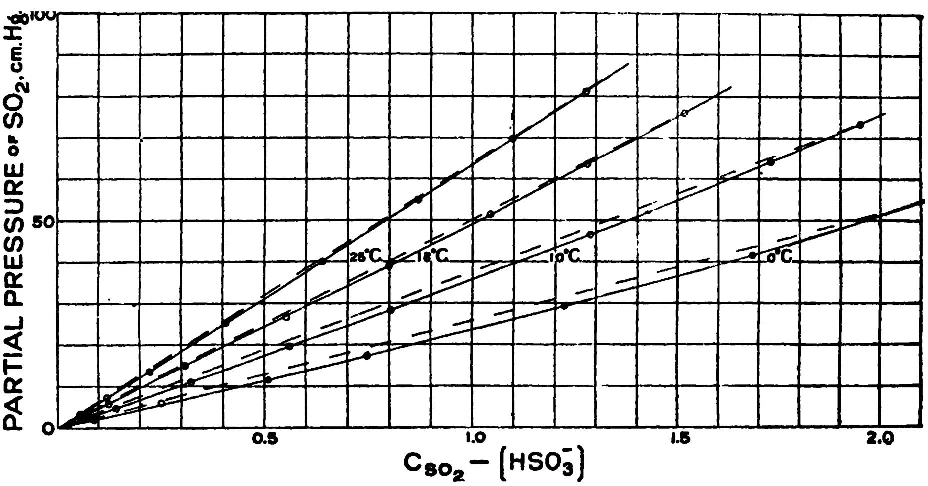


FIG. 4. *Relation of* $C_{SO_2} - [HSO_3^-]$ *to partial pressure of sulphur dioxide.*

The accompanying figure will show that the approximation due to Campbell and Maass (8) does not hold at the lower temperatures and with the more accurate data. When $C_{SO_2} - [HSO_3^-]$ is plotted against the partial pressure of sulphur dioxide above the solution, a decided curvature is noted, which increases with decreasing temperature as shown by the straight dotted lines.

This cannot possibly be due to experimental error, which will be seen from the following figures taken from the above curves drawn on a large scale. At a concentration of one gm.-mol per litre the experimental error would have to be 2 cm. in a measurement of 20 cm. whereas the experimental results are correct to 0.1 mm., or an error of 10% in the estimated value of the concentration, whereas 0.1% is the probable maximum error. Especially when it is seen that all the curves as given by the experimental points have a consistent curvature shown by their continuity, it is realized that the straight line relationship is one which will only hold at high temperatures.

It is possible to predict this curvature from the equation

$$C_{SO_2} = Hp\left(1+\frac{[H_2O]}{K_1}\right) + [HSO_3^-]$$

With increasing temperature K_1 increases rapidly and with increasing sulphur dioxide concentration $[H_2O]$ decreases. The combined effect of these two factors tends to diminish the value of $1 + \frac{[H_2O]}{K_1}$ and at an infinitely high concentration and temperature this term would become unity and the equation would become that of a straight line. This proves that had the work of Campbell and Maass (8) been sufficiently accurate they would have obtained curved plots at their lower temperatures and approximations to straight lines at the higher temperatures. However, as has been stated before, their water concentration did not vary enough to warrant this.

In the evaluation of the constants H and K_1 in the present work, equations (10) and (11), due to C. Maass (31), were taken as a starting point.

$$C_{H_2O} = [H_2O] + [H_2SO_3] + [HSO_3^-] \tag{10}$$

$$C_{SO_2} = [SO_2] + [H_2SO_3] + [HSO_3^-] \tag{11}$$

From (11)

$$C_{SO_2} = Hp\left(1+\frac{[H_2O]}{K_1}\right) + [HSO_3^-] \text{ (or } [H^+])$$

$$C_{SO_2} - [H^+] = Hp\left(1+\frac{[H_2O]}{K_1}\right)$$

$$= Hp\left(\frac{1+C_{H_2O} - C_{SO_2} + Hp}{K_1}\right)$$

For convenience let $C_{SO_2} - [H^+] = a$, and $C_{H_2O} - C_{SO_2} = b$

Then

$$a = Hp\left(1+\frac{b+Hp}{K_1}\right) \tag{16}$$

The only unknowns in equation (16) are H and K_1. It was transformed into a quadratic and by inserting values for the other terms, a, b, and p, in two such equations values for H and K_1 were calculated. From this calculation values of H were found to be very small and negative. It was quite obvious that H must be small and positive and that the negative value obtained could be attributed to small experimental errors which were just large enough to transform H from a small positive to a small negative quantity.

After a careful consideration of the situation the following procedure was adopted.

$$a = Hp\left(1+\frac{b+Hp}{K_1}\right) \tag{17}$$

$$A = HP\left(1+\frac{B+HP}{K_1}\right) \tag{18}$$

Equations (17) and (18) represent two points on a sulphur dioxide isothermal. From previous calculations it was concluded that the value of Hp was small

compared with b. Hence, for the time being, Hp was disregarded in the term $b+Hp$ and the equations became

$$a = Hp\left(1+\frac{b}{K_1}\right) \tag{19}$$

$$A = HP\left(1+\frac{B}{K_1}\right) \tag{20}$$

Divide (19) by (20)

$$\frac{a}{A} = \frac{p\left(1+\frac{b}{K_1}\right)}{P\left(1+\frac{B}{K_1}\right)}$$

By letting $\frac{a}{p} = d$ and $\frac{A}{P} = D$ and rearranging it follows that

$$K_1 = \frac{b\left(\frac{B}{b} - \frac{D}{d}\right)}{\frac{D}{d} - 1} \tag{21}$$

By substituting the value of K_1 obtained in (21) in equations (19) and (20) it was possible to calculate H.

There was considerable incoherent variation in K_1 and it appeared to be very sensitive to small errors in experimental data. This was overcome to a large extent by plotting per cent sulphur dioxide against d for each temperature considered and by inspection picking out pairs of logical d points from which to calculate K_1. An average of the values for K_1 thus obtained at 25 and 18° C., when substituted in equation (16), permitted of a calculation of "a" (*i.e.*, $C_{SO_2} - [H^+]$) within two or three per cent. By trial and error, *i.e.*, by varying slightly the values of H and K_1, the correct values of "a" were obtained within 0.2%. In this way the validity of the constants H and K_1 at 25 and 18° C. was established.

This work was led further afield than was originally expected. At 10° C. the average approximate value of K_1, as calculated from (21), was found to be -2.6, and at 0° C. it was -12.1. Equation (16) may be transformed to

$$K_1 = \frac{Hpb - H^2p^2}{a - Hp} \tag{22}$$

In equation (22) H^2p^2 is small with respect to Hpb. H is a constant and at any given concentration and temperature p is a constant, hence an unusually small value of K_1 would be caused by an excessive diminution of the value of b. Since b is the value of the apparent concentration of the water, a low value of b would indicate that water was being removed by some means and would point toward hydrate formation. This is in agreement with the fact that below 12.1° C. a stable heptahydrate forms. In all that has gone before the influence of hydrate formation and hydration of ions on water concentration has been neglected. The influence of the solute on the equilibria, excepting between the various associated water molecules, has also been neglected. It is necessary to assume that these factors are outweighed by those which have been taken into account and emphasis is placed by the authors on the approximate nature of all the calculations. Hydration increases with lower temperature and

evidently becomes very marked at 12.1° C. by the separation of a heptahydrate and is also indicated by the above considerations to be of such an order of magnitude that it cannot be neglected for the 10 and 0° C. data.

In order to determine the extent of the hydrate formation a new equilibrium must be considered, *i.e.*, between the hydrate, the sulphur dioxide, and the water:

$$SO_2 + nH_2O \rightleftharpoons SO_2.nH_2O$$

$$[SO_2]\,[H_2O]^n = \alpha[SO_2.nH_2O] \tag{23}$$

where n is the number of molecules of water in the hydrate. The equilibrium

$$[SO_2]\,[H_2O] = K_1[H_2SO_3]$$

must also be kept in mind.

As a starting point

$$C_{SO_2} = [SO_2] + [H_2SO_3] + [H^+] + [SO_2\,aq.] \tag{24}$$

$$C_{H_2O} = [H_2O] + [H_2SO_3] + [H^+] + n[SO_2\,aq.] \tag{25}$$

$$C_{HO_2} - C_{SO_2} = [H_2O] - [SO_2] + (n-1)\,[SO_2\,aq.]$$

$$b = [H_2O] - [SO_2] + \frac{(n-1)\,[SO_2]\,[H_2O]^n}{\alpha}$$

$$= [H_2O] - Hp + \frac{(n-1)Hp\,[H_2O]^n}{\alpha} \tag{26}$$

Also from equation (24)

$$C_{SO_2} = Hp + [H^+] + \frac{[H_2O]Hp}{K_1} + \frac{[H_2O]^nHp}{\alpha}$$

and

$$C_{SO_2} - [H^+] = Hp\left(1 + \frac{[H_2O]}{K_1} + \frac{[H_2O]^n}{\alpha}\right)$$

For convenience let $[H_2O] = [x]$, then

$$a = Hp\left(1 + \frac{[x]}{K_1} + \frac{[x]^n}{\alpha}\right) \tag{27}$$

or

$$a - Hp - \frac{[x]Hp}{K_1} = \frac{Hp[x]^n}{\alpha} \tag{28}$$

From (26)

$$b + Hp - [x] = \frac{(n-1)Hp[x]^n}{\alpha} \tag{29}$$

and combining (28) and (29)

$$\frac{b + Hp - [x]}{n-1} = a - Hp - \frac{[x]Hp}{K_1} \tag{30}$$

The value of [x] may be calculated from equation (30) provided that the values of H and K_1 are known at 10° C. Furthermore the numerical value of 7 for n may or may not be legitimate. What equation (30) really represents is that water is removed from active participation in the equilibrium in ways

other than the straightforward H_2SO_3 formation. Whether water is used up in the hydration of the ions is a moot question. Equation (30) in which n is given the somewhat arbitrary value of 7 can therefore only be looked upon as a crude approximation, valuable however in showing that another factor has to be taken into account.

What has been said above has to be borne in mind in all the calculations involving equation (30). All that can be hoped for is that reasonable values of H, K_1 and α will satisfy the experimental results. At higher temperatures also hydrate formation probably has its influence, though the higher the temperature the smaller will be its effect.

The values of H and K_1 were known for 18 and 25° C. and it was a debatable question whether a straight line extrapolation to 10 and 0° C. would be legitimate. On investigating the constants similar to H for inert gases, as given in the literature, it was found that they did not vary with temperature according to a straight line relationship. Reference was made to the apparent H calculated for sulphur dioxide from the relation $H = \frac{a}{p}$. This, of course, exhibited curvature when plotted against temperature. An arbitrary value of H at 10° C. was obtained by calculating a variation of the real H corresponding to the variation of the apparent H. To obtain the corresponding K_1 the ratios of $\frac{H}{K_1}$ were calculated at 18 and 25°C. and were extrapolated to 10°C. Since H had already been determined it was possible to calculate K_1.

Using these values obtained for H and K_1 the corresponding values of [x] at 10° C. were calculated. The values of α were then determined by inserting these approximations in equation (29) and an average value of α was obtained. These values were remarkably constant. Then, by supplying the necessary values in equation (27) it was possible to calculate values of "a" very accurately, there being only an average mean deviation of 0.1% from the experimental values.

The real dissociation constant

$$K_2 = \frac{[H^+]\ [HSO_3^-]}{[H_2SO_3]}$$

was calculated as follows:

$$K_1 = \frac{[H_2O]\ [SO_2]}{[H_2SO_3]} \tag{4}$$

On rearranging and substituting

$$[H_2SO_3] = \frac{[H_2O]\ [SO_2]}{K_1} = \frac{(b+Hp)\ (Hp)}{K_1}$$

Hence

$$K_2 = \frac{K_1[H^+]^2}{(b+Hp)\ (Hp)}$$

K_2 is calculated only at 18 and 25° C. since the 10° C. calculations, as pointed out above, are the result of only arbitrary approximations.

The theoretical data calculated in connection with this work are presented in Table II for temperatures of 0, 10, 18, and 25° C. C_{SO_2} is the total amount

TABLE II

THEORETICAL DATA CALCULATED FOR THE SYSTEM SULPHUR DIOXIDE-WATER

SO_2, %	Density	C_{SO_2}	C_{H_2O}	Specific cond. $\times 10^3$	$[HSO_3^-]$	a	b	$K_a \times 10^3$	$K_1 \times 10^3$	a(calc.)
At 0° C.										
0.1369	1.0004	0.0213	55.46	3.39	0.0135	0.0078	55.44	23.47		
0.3374	1.0015	0.0523	55.40	6.50	0.0259	0.0263	55.35	25.59		
0.8888	1.0048	0.1393	55.26	12.53	0.0500	0.0893	55.12	28.00		
2.284	1.0127	0.3608	54.92	22.55	0.0900	0.2708	54.56	29.91		
3.978	1.0223	0.6342	54.46	31.35	0.1251	0.5091	53.83	30.74		
5.597	1.0317	0.9005	54.05	38.00	0.1517	0.7488	53.15	30.72		
8.630	1.0488	1.412	53.18	48.04	0.1917	1.221	51.77	30.11		
11.52	1.0604	1.905	52.08	55.60	0.2219	1.683	50.18	29.26		
14.23	1.0663	2.367	50.76	61.58	0.2458	2.123	48.40	28.46		
At 10° C.										
0.1267	1.0001	0.0197	55.43	3.66	0.0117	0.0080	55.41	17.26		—
0.3414	1.0012	0.0558	55.39	7.48	0.0240	0.0318	55.34	18.13		0.029
0.6360	1.0028	0.0995	55.29	11.36	0.0365	0.0629	55.19	21.13		0.063
1.261	1.0061	0.1978	55.12	17.59	0.0564	0.1414	54.93	22.55		0.139
2.593	1.0131	0.4097	54.75	27.27	0.0875	0.3222	54.34	23.78		0.320
4.256	1.0219	0.6784	54.30	36.38	0.1168	0.5616	53.62	24.29		0.561
5.872	1.0305	0.9436	53.80	43.55	0.1398	0.8038	52.86	24.32		0.804
8.927	1.0465	1.459	52.85	54.31	0.1744	1.285	51.39	23.65		1.286
11.68	1.0575	1.926	51.82	61.89	0.1986	1.728	49.90	22.84		1.732
13.03	1.0613	2.156	51.22	64.91	0.2084	1.948	49.07	22.28		1.954
At 18° C.										
0.0816	0.9988	0.0127	55.39	2.96	0.0083	0.0044	55.38	15.53	17.52	
0.2605	0.9997	0.0406	55.35	6.89	0.0192	0.0214	55.31	17.31	27.64	
0.5563	1.0012	0.0868	55.25	11.43	0.0319	0.0549	55.16	18.57	31.29	
1.103	1.0040	0.1727	55.11	17.57	0.0491	0.1236	54.94	19.48	33.58	0.1232
2.458	1.0108	0.3877	54.71	28.32	0.0791	0.3086	54.32	20.27	35.18	0.3069
4.150	1.0195	0.6594	54.19	38.00	0.1061	0.5533	53.53	20.36	35.69	0.5476
5.773	1.0278	0.9255	53.74	45.56	0.1273	0.7982	52.82	20.29	35.46	0.7958
7.340	1.0357	1.186	53.26	51.69	0.1444	1.042	52.08	20.01	35.02	1.042
8.831	1.0432	1.436	52.76	56.67	0.1582	1.278	51.32	19.60	34.43	1.281
10.30	1.0498	1.687	52.28	61.08	0.1706	1.516	50.59	19.20	33.91	1.520
At 25° C.										
0.1735	0.9978	0.0270	55.27	5.45	0.0137	0.0133	55.24	14.08	27.48	
0.5448	0.9996	0.0849	55.20	11.74	0.0295	0.0554	55.12	15.70	29.49	
1.056	1.0010	0.1649	54.96	17.75	0.0446	0.1203	54.80	16.52	31.52	
1.809	1.0059	0.2838	54.81	24.43	0.0613	0.2225	54.53	16.91	32.40	0.2194
3.116	1.0123	0.4918	54.42	33.55	0.0842	0.4076	53.93	17.41	33.18	0.4055
4.672	1.0200	0.7433	53.96	42.01	0.1045	0.6379	53.22	17.45	33.33	0.6379
6.184	1.0275	0.9906	53.47	48.66	0.1222	0.8684	52.48	17.19	32.83	0.8720
7.640	1.0346	1.233	53.04	54.16	0.1360	1.097	51.81	16.86	32.37	1.102
8.756	1.0403	1.420	52.66	57.69	0.1449	1.275	51.24	16.46	31.92	1.273

of sulphur dioxide in the solution expressed in gram-mols per litre. C_{H_2O} is the concentration of the water in the solution also expressed in gram-mols per litre. The density values are those obtained by Campbell and Maass (8). $[HSO_3^-]$ is the concentration of the HSO_3^- ion and is obtained by dividing the specific conductivity by the limiting value given in the following table for the conductivity of H_2SO_3 and multiplying by 1000 to bring it to gram-mols per litre. The values for the limiting conductivity of the HSO_3^- ion were obtained from the formula of Kohlrausch (26).

$$u_t = 44.3\{1 + 0.0242(t-18) + 0.00011(t-18)^2\}$$

using Kerp and Baur's (23) value at 25° C. as revised by Lindner (28). The values for the H^+ ion of course have been well established.

TABLE III

LIMITING CONDUCTIVITIES OF SULPHUROUS ACID

Temp., °C.	H^+	HSO_3^-	H_2SO_3	Temp., °C.	H^+	HSO_3^-	H_2SO_3
0	224.2	26.4	250.6	18	315.0	44.3	359.3
10	275.5	36.0	311.5	25	348.5	49.7	398.2

The values given for a are those of $C_{SO_2} - [HSO_3^-]$ and represent the undissociated sulphurous acid providing that all the sulphur dioxide is combined with water. $C_{H_2O} - C_{SO_2}$ is the b value given and represents the amount of uncombined water assuming that all the sulphur dioxide is combined with water. These assumptions are not true but this mode of tabulation serves as a convenient means in the calculations. The calculation of K_a, K_2 and a has been previously discussed.

The equilibria constants have been obtained more accurately than ever before and fit in with the experimental data within the range of experimental error. The results of previous experimenters have failed to show definitely various fine points which are quite important. For instance, Campbell and Maass (8), Lindner (28), and Kerp and Baur (23) noted that the value of the apparent dissociation constant, K_a, varied with temperature but they did not notice any regular variation with changing concentration. Fig. 5 shows that there is a distinct variation of K_a at all temperatures considered, the rate of

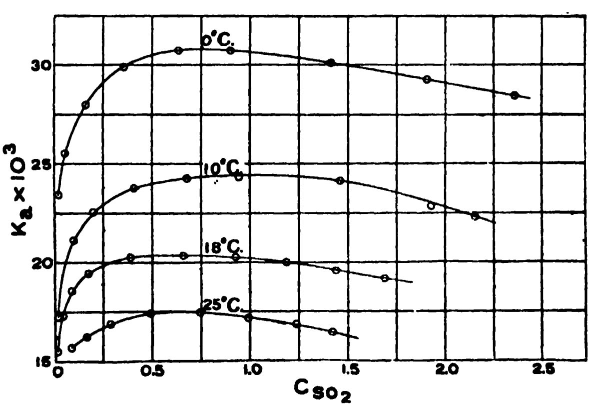


FIG. 5. *Relation of K_a to concentration in the system sulphur dioxide-water.*

increase being greater at the lower concentrations. With increasing temperatures there is decreasing variation. This may be in agreement with the theory evolved above provided that another factor, only important at very low concentrations, is taken into account. This new factor is the secondary dissociation of the sulphurous acid, the additional H^+ ion causing an increase in K_a.

The equilibrium

$$H_2O + SO_2 \rightleftarrows H_2SO_3$$

has the opposite effect causing a decrease in K_a with rise in concentration as given by the equation

$$K_a = \frac{K_2[H_2O]}{K_1+[H_2O]}$$

Since the secondary dissociation loses its effect with increasing concentration a maximum value of K_a must be reached. Since K_1 increases with rise in temperature the rate of decrease in K_a beyond the maximum concentration should be less at the higher temperatures which was pointed out above. Furthermore it can be predicted that the maximum value of K_a is shifted to a higher concentration with rise in temperature. This variation in K_a is therefore one of the best proofs of the existence of uncombined sulphur dioxide, and also that the amount of free sulphur dioxide increases with rise in temperature.

In the comparison of the observed values of a with those calculated on the basis of equation (16) it is apparent that the poorest agreement is found at the very low concentrations. This is due to the secondary dissociation which has not been taken into account.

The value of K_a reaches a maximum and remains practically constant over a short range of concentration but it then begins to fall off again. For this reason it is illegitimate to strike an average value. The maximum values agree very well with the average values of previous experimenters. Comparison is only possible at 0 and 25° C.

TABLE IV

COMPARISON OF APPARENT DISSOCIATION CONSTANTS

Temperature, °C.	Kerp and Baur	Lindner	Campbell and Maass	This work
0		31.1	31.3	30.7
25	17.4	17.4	17.4	17.4

Dissociation constants do not ordinarily vary to any great extent with temperature. The large variation of K_a proves that it is only an apparent dissociation constant. The values of K_2 at 18 and 25° C., which vary only slightly, prove that it is a true dissociation constant. K_2 varies with the concentration to about the same extent that K_a does. This follows from what has gone before. It is evident that K_2 will always be larger than K_a but will approach it in value when K_1 is small.

Table V contains the values for H and K_1 at the three temperatures investigated.

TABLE V

H AND K_1 AT VARIOUS TEMPERATURES

Temperature, °C.	10	18	25
H	0.01080	0.00870	0.00758
K_1	36.80	39.50	48.51

It will be noted that the value of H decreases and that of K_1 increases with rising temperature. This is what would be expected from the respective equilibria

$$[SO_2] = Hp \tag{2}$$

and

$$K_1 = \frac{[H_2O]\ [SO_2]}{[H_2SO_3]} \tag{4}$$

From equation (2) the per cent of uncombined sulphur dioxide can be readily calculated. The values given in Table VI show their order of magnitude.

TABLE VI

COMPARISON OF UNCOMBINED SULPHUR DIOXIDE CONCENTRATION WITH TOTAL SULPHUR DIOXIDE CONCENTRATION

		2	4	6	8	10
C_{SO_2}, %		2	4	6	8	10
SO_2, %	18° C.	0.626	1.407	2.221	3.071	3.962
	25° C.	0.732	1.608	2.573	3.457	—

The constants H, K_1, and K_2 have not been evaluated for the data at 0° C. It was deemed sufficient proof for the theory to have consistent data at three temperatures. The calculations at 10° C. were very lengthy and it was foreseen that it would take even longer to handle the data at 0° C. since it would necessitate finding values for H and K_1 by trial. The extrapolation of the values of H and K_1 from 10 to 0° C. could not be expected to follow the same ratio as from 18 to 10° C.

After a discussion of the carbon dioxide and ammonia systems the conclusions to be drawn from the sulphur dioxide system will be summed up once more.

Carbon Dioxide

An investigation of the equilibria existing in aqueous solutions of carbon dioxide presents essentially different problems from those encountered in sulphur dioxide systems. Sulphur dioxide has a solubility in water sixty times greater than that of carbon dioxide. Sulphurous acid behaves as a strong acid while carbonic acid is apparently very weak and has an extremely small apparent dissociation constant. For these reasons the equilibria must be investigated from a different standpoint as will be seen in this section.

Experimental

The carbon dioxide was generated in a Kipp loaded with very pure marble and dilute hydrochloric acid. The gas was passed through a water wash bottle to remove any acid spray and then through two more wash bottles, one containing alkaline potassium permanganate and the other a solution of sodium carbonate. It was then passed slowly through a large phosphorus pentoxide tube and was condensed with liquid air. This was followed by a triple distillation similar to the one discussed in the experimental section.

The only possible impurity in the gas was a trace of hydrogen chloride. A test was made for this by bubbling a sample of the gas through a solution of silver nitrate. After half an hour no precipitation of silver chloride was noted, which was considered sufficient evidence that no hydrogen chloride was present.

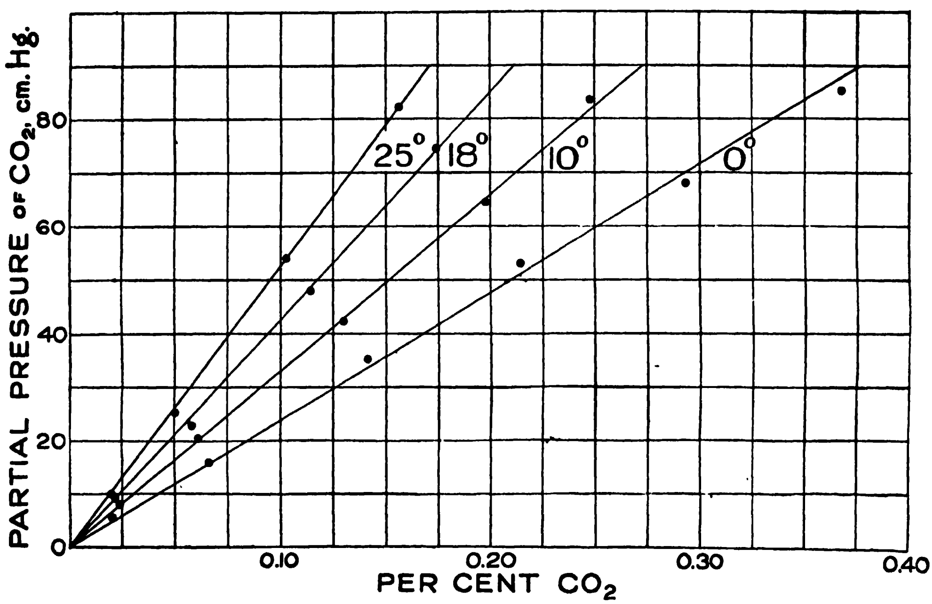


FIG. 6. *Partial vapor pressures of carbon dioxide solutions.*

Due to the fact that carbon dioxide possesses such a small solubility in water every precaution had to be taken in the measurement of the amount of gas injected. The rate of solution was very slow and a long time was necessary to reach equilibrium, usually about 90 min. After completing a run at 0° C. it was decided that much time would be saved if solutions were prepared at 10° C. and after making measurements raise the temperature to 18° and 25° C. since equilibrium was reached more rapidly when the gas was coming out of the solution than when it was going into solution. This procedure was found to be much more satisfactory.

Results

Table VII contains the experimental data and the theoretical data derived from them. The units are the same as those used for sulphur dioxide. Fig. 6 presents the vapor pressure relationships and Fig. 7, the specific conductivity.

It should be stated at this point that the vapor pressure data at 0° C. are inaccurate. The plot in Fig. 6 would indicate that the evacuated arm of the manometer had sprung a leak at some time after a concentration of 0.25% carbon dioxide had been reached since the vapor pressure as measured was too low. Hence the data at 10, 18 and 25° C. only will be considered in the discussion.

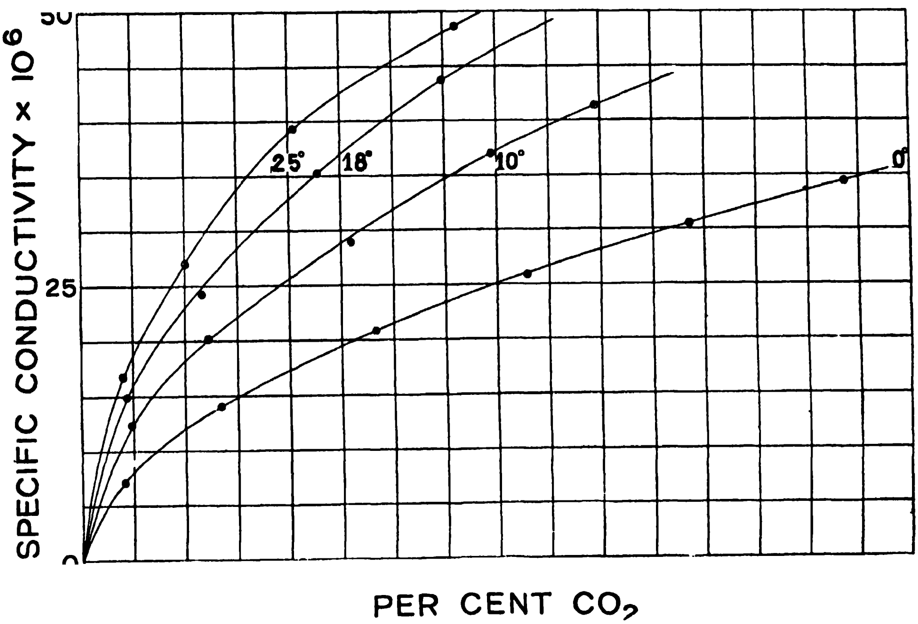


FIG. 7. *Specific conductivity of carbon dioxide solutions.*

The vapor pressure and conductivity data at 10, 18 and 25° C. were consistent within themselves and the curves show the regularity that is to be desired. It will be noticed that the maximum concentration reached at 0° C. was 0.36% as compared with a maximum concentration of 14.2% for sulphur dioxide. In this region of concentration the vapor pressure of both gases is approximately atmospheric. This gives a measure of the relative solubilities.

Since carbon dioxide solutions having vapor pressures of one atmosphere or less are of very low concentration, in every case less than 0.5%, there is only a very slight change in the water concentration, in fact it may be considered as being constant. Hence it has not been possible to treat the equilibria in the same manner as those of sulphur dioxide.

TABLE VII

EXPERIMENTAL AND THEORETICAL DATA FOR THE SYSTEM CARBON DIOXIDE-WATER

CO_2 %	C_{CO_2}	Specific cond. $\times 10^6$	Partial pressure of CO_2, cm. Hg.	$[H^+]$	a	$K_a \times 10^8$	$H \times 10^5$
At 0° C.							
0.0202	0.00458	7.19	5.70	0.000028	0.00456	17.44	80.00
0.0661	0.01503	14.02	16.00	0.000055	0.01497	20.18	93.58
0.1412	0.03208	20.89	35.27	0.000082	0.03200	20.93	90.72
0.2143	0.04870	25.90	53.02	0.000101	0.04860	21.23	92.06
0.2929	0.06656	30.57	68.13	0.000119	0.06640	21.65	97.47
0.3676	0.08354	34.42	85.30	0.000135	0.08341	21.85	97.81
							Av. 91.94
At 10° C.							
0.0240	0.00545	12.30	8.17	0.000038	0.00541	26.87	66.22
0.0611	0.01389	20.18	20.61	0.000062	0.01383	28.30	67.11
0.1297	0.02947	29.89	42.41	0.000092	0.02938	29.22	69.28
0.1975	0.04488	37.00	64.48	0.000115	0.04477	29.39	69.45
0.2475	0.05624	41.34	83.56	0.000128	0.05611	29.27	67.16
							Av. 67.84
At 18° C.							
0.0216	0.00491	14.92	9.23	0.000039	0.00487	32.48	52.82
0.0580	0.01318	24.80	22.98	0.000066	0.01312	33.35	57.10
0.1140	0.02590	35.25	49.14	0.000094	0.02581	34.23	52.52
0.1740	0.03954	43.69	74.45	0.000116	0.03942	34.44	52.94
							Av. 53.84
At 25° C.							
0.0200	0.00454	16.71	9.89	0.000040	0.00450	35.22	45.50
0.0502	0.01141	27.00	25.32	0.000064	0.01135	36.46	44.83
0.1025	0.02329	39.33	54.00	0.000093	0.02320	37.84	42.96
0.1559	0.03542	48.64	82.22	0.000116	0.03530	38.04	42.93
							Av. 44.05

Consider an equation of the form of (13a) in connection with carbon dioxide

$$C_{CO_2} = Hp\left(1 + \frac{[H_2O]}{K_1}\right) + [H^+]. \tag{31}$$

In this instance the value of H_2O is constant, hence the term $\left(1 + \frac{[H_2O]}{K_1}\right)$ is a constant. Equation (31) is, therefore, that of a straight line. When $C_{CO_2} - [H^+]$ was plotted against the partial pressure of carbon dioxide a straight line relationship was obtained as predicted. This is illustrated in Fig. 8. If concentrations of carbon dioxide as high as 5 to 10% were obtained there would be sufficient change in the water concentration to make the above

approximation illegitimate and a plot, similar to that given in Fig. 8 would show curvature such as is exhibited in Fig. 4 for sulphur dioxide. Such a procedure would necessitate the measurement of vapor pressures up to approximately 100 atmos. Vapor pressures of this order of magnitude have been measured by Sander (43) but with insufficient accuracy for this purpose and no conductivity data at these pressures are available.

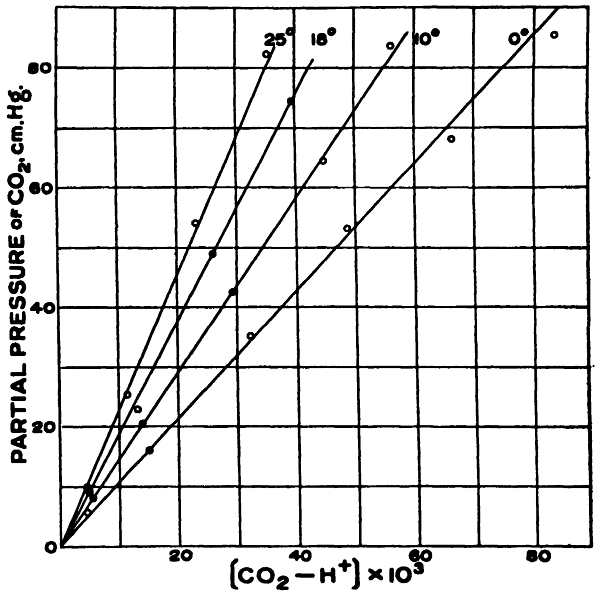


FIG. 8. *Relation of* $C_{CO_2} - [H^+]$ *to partial pressure of carbon dioxide.*

In Table VIII, P_S is the vapor pressure in atmospheres as obtained by Sander (43) experimentally. The column P_H contains the pressures corresponding to the concentrations in the first column calculated using the Henry's Law constant (1.105×10^6) obtained in the present work.

TABLE VIII

COMPARISON OF CARBON DIOXIDE VAPOR PRESSURE DATA DUE TO SANDER WITH THEORETICAL DATA CALCULATED FROM HENRY'S LAW

C_{CO_2}	0.7278	0.8974	1.067	1.199
P_S	25	35	45	53
P_H	18.8	23.1	27.4	30.1

It would be expected that if the values of P_S were plotted against concentration they would exhibit curvature. On the other hand they give a straight line relationship, curvature if any being not discernible. This straight line when extrapolated does not pass through the origin as it theoretically should but meets the pressure axis at a point corresponding to −18 atmospheres. The improbability of Henry's Law holding at these high pressures combined with the peculiar results obtained from extrapolation indicates that Sander's (43) data are inaccurate and show additional reason for continuing this work at higher pressures.

The apparent dissociation constant has been calculated from the relationship

$$K_a = \frac{[H^+]\,[\tfrac{1}{2}CO_3^-]}{C_{CO_2} - [H^+]}.$$

The limiting value of the conductivity of the $\frac{1}{2}CO_3^-$ ion was obtained at the various temperatures from the value given by Kohlrausch (26) at 18° C. by employing the equation

$$T = 60 \left\{1 + 0.0270\ (T - 18)\right\}$$

The values are given in Table IX.

TABLE IX

IONIC MOBILITIES OF CARBONIC ACID (KOHLRAUSCH)

Temp., °C.	H^+	$\frac{1}{2}CO_3^-$	H_2CO_3	Temp., °C.	H^+	$\frac{1}{2}CO_3^-$	H_2CO_3
0	224.2	30.8	255.0	18	315.0	60.0	375.0
10	275.5	47.0	322.5	25	348.5	71.3	419.8

The concentration of the H^+ ion was calculated by the same method as HSO_3^- in the previous section.

The constant H calculated from the values of $\frac{a}{p}$ is the reciprocal of the Henry's Law constant given in the International Critical Tables, the value of a in this case being in gram-mols per litre rather than in mol fraction as in the tables.

The vapor pressure data agree very well with those of Bohr (5). This agreement is indicated in the tabulation of Henry's Law constants given in Table X. In this case

$$H_1 = \frac{\text{partial pressure of } CO_2 \text{ in mm. of Hg.}}{\text{mol fraction of } CO_2}$$

TABLE X

HENRY'S LAW CONSTANTS FOR CARBON DIOXIDE

Temperature, °C.		10	18	25
Henry's law constants	Bohr	0.791	1.018	1.243
	Present work	0.797	1.039	1.255

It will be noted that in each case the present work gives a slightly higher value for the constant, the difference being fairly regular.

The conductivity data agree almost perfectly with those of Knox (24) and fairly well with those of Pfeiffer (42), but are constantly higher than those of Kendall (22). This is brought out in Table XI which compares the present work with that of Knox (24) and Kendall (22) at 18° C.

Data at other temperatures where comparison is possible are not tabulated since the same percentage deviation is noted in each case.

A good part of this deviation may be accounted for in Kendall's determination of the carbon dioxide concentration. Due to the fact that it is possible to prepare solutions of only very low concentrations at the pressures here

TABLE XI

SPECIFIC CONDUCTIVITY OF CARBONIC ACID AT 18° C.

C_{CO_2}	Specific conductivity $\times 10^6$		
	Kendall	Knox	Present work
0.0080	17.30	19.56	19.00
0.0188	27.20	29.70	29.69
0.0400		43.77	43.80

considered, a small error in the determination of this concentration would cause a large percentage error. Kendall, using a modification of Pettenkofer's method (48), ran the solution directly from the cell into a bottle containing air free from carbon dioxide. Excess of baryta solution of known concentration was added and the carbon dioxide was determined as barium carbonate. For larger concentrations of carbon dioxide this would be considered by the writers as a sound procedure but in this case it verges on microanalysis. An average concentration of carbon dioxide as determined by Kendall (22) was 0.02 gram-mols per litre. Working with 30 cc. of such a solution, as he did, meant the absolute determination of approximately 0.003 gm. of barium carbonate by titrating the excess of barium hydroxide with hydrochloric acid. Kendall stated that his determinations checked within 0.2% but this would not mean that his determination was correct within that amount as far as the absolute value is concerned. A rough calculation shows that his possible error is in the region of 5%.

In the present work the amount of gas added to the solution was measured by taking pressure and temperature readings of the gas in a calibrated volume before injecting it into the reaction chamber. This combined with vapor pressure readings good to 0.02 cm. permitted of the calculation of the concentration with an accuracy of 0.1%.

A comparison of the conductivity data due to Kendall (22) and those of the present work may also be obtained from Table XII which contains the dissociation constants, maximum values being given in each case.

TABLE XII

DISSOCIATION CONSTANTS OF CARBONIC ACID

Temperature, °C.		18	25
Dissociation constants $\times 10^8$	Kendall	31.4	34.9
	Present work	34.4	38.0

Another point which is not brought out in Kendall's work is the regular variation of K_a with concentration as is shown in Fig. 9. The values of Knox (24), on the other hand, show this variation. This would indicate more

precise measurements in the present work. Kendall also claims that his K_a is a true dissociation constant but previous discussions in this paper have proved that it is only an approximate constant.

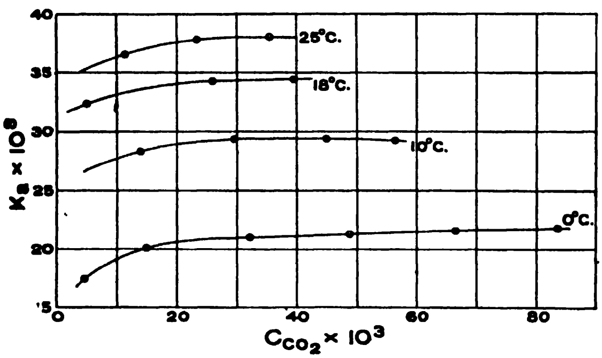


FIG. 9. *Relation of K_a to concentration in the system carbon dioxide-water.*

As has been previously stated, such a small range of concentration permits of only an approximate determination of the equilibrium existing in solutions of carbon dioxide. This work has been merely preliminary and it is hoped that it may be continued by future workers who are interested in this type of research. The value of such data as would be obtained has been previously discussed.

AMMONIA

The system ammonia-water differs in several ways from the two previous systems studied. For vapor pressures of corresponding magnitudes ammonia is two hundred times more soluble in water than is sulphur dioxide. The specific conductivity of ammonia solutions rises sharply to a maximum at low concentrations and then decreases rapidly becoming asymtotic to zero at very

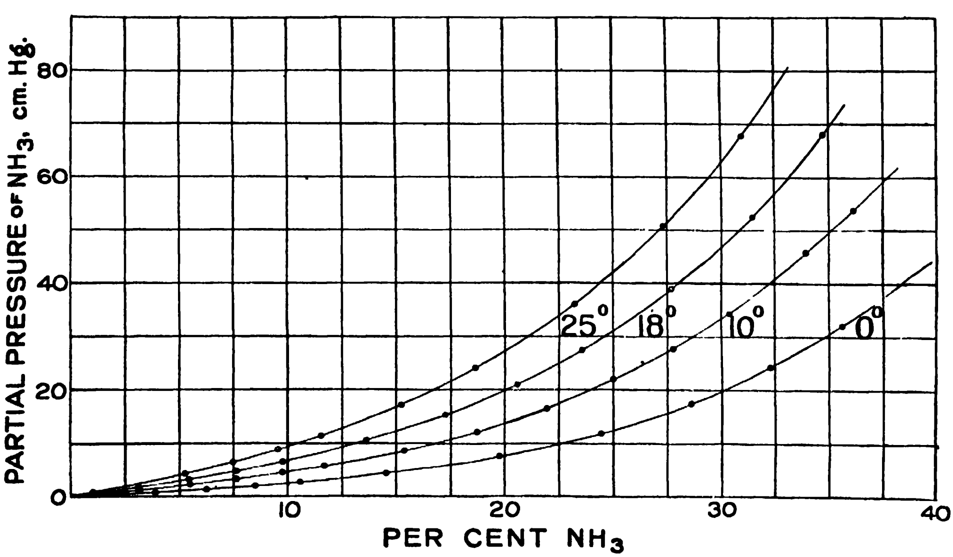


FIG. 10. *Partial vapor pressures of ammonia solutions.*

high concentrations. At the concentrations covered for sulphur dioxide and carbon dioxide the conductivity rose steadily and there was not much indication of a maximum being reached.

A cylinder of liquid ammonia was obtained from the Matheson Company, North Bergen, N.J. Freezing point and vapor pressure determinations showed it to be very pure, there being only a trace of water present. Purification was effected by passing the ammonia, in the gaseous state, through long tubes of

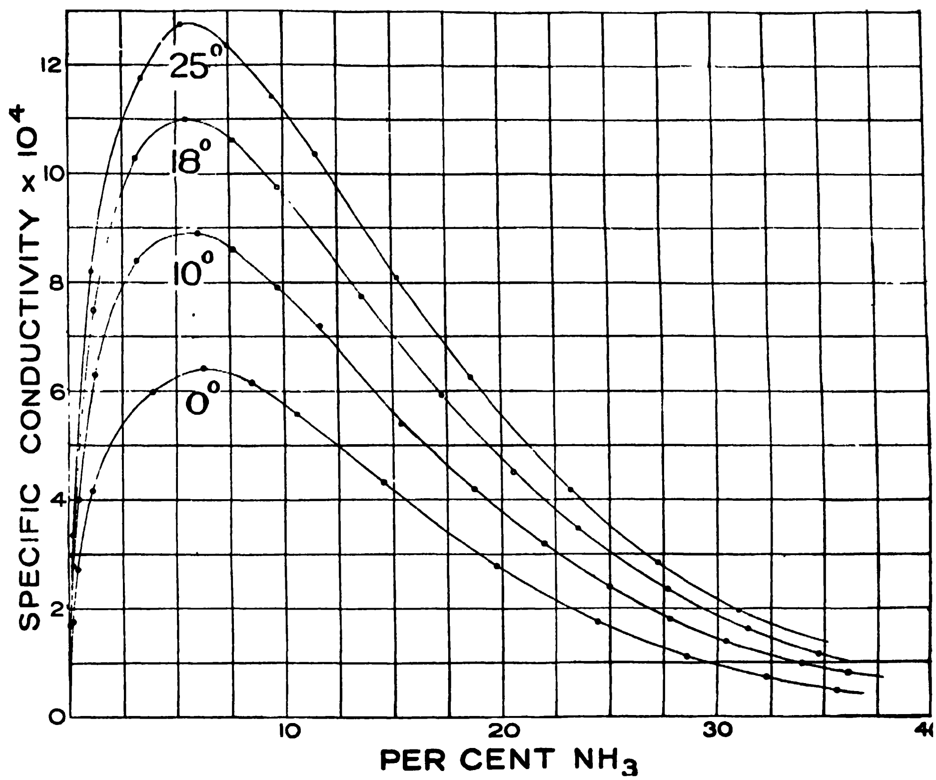


FIG. 11. *Specific conductivity of ammonia solutions.*

lime and condensing it again with a dry ice-ether mixture. The drying process was followed by two fractional distillations, the middle fraction (75%) being retained. The solutions were prepared as previously discussed in the experimental section. The conductivity cell allowing for 100% expansion of the solution was used.

The experimental data are given in Table XIII for temperatures of 0, 10, 18, and 25° C. Fig. 10 and 11 present the vapor pressure and specific conductivity data relationships at the four temperatures. The lower vapor pressures, where the change in pressure with increasing concentration is very small, were measured with a cathetometer. In the calculation of the concentrations the molecular weights of ammonia as found by Carpenter (9)

TABLE XIII

EXPERIMENTAL DATA OBTAINED FOR THE SYSTEM AMMONIA-WATER

NH_3 %	Density	C_{NH_3}	Specific cond. $\times 10^4$	Partial pressure of NH_3, cm. Hg.	NH_3 %	Density	C_{NH_3}	Specific cond. $\times 10^4$	Partial pressure of NH_3, cm. Hg.
AT 0° C. Weight of water used = 21.124 gm.									
0.419	0.9984	0.246	2.721	0.15	14.51	0.9482	8.095	4.327	4.46
1.072	0.9958	0.628	4.167	0.29	19.76	0.9324	10.84	2.791	7.75
3.882	0.9847	2.249	5.992	0.70	24.42	0.9191	13.20	1.754	12.05
6.241	0.9759	3.583	6.421	1.43	28.58	0.9078	15.26	1.138	17.71
8.474	0.9682	4.827	6.152	2.00	32.28	0.8983	17.06	0.741	24.38
10.56	0.9610	5.970	5.584	2.72	35.60	0.8900	18.64	0.488	32.18
At 10° C. Weight of water used = 21.525 gm.									
0.213	0.9990	0.125	2.732	0.10	15.35	0.9424	8.511	5.373	8.82
0.448	0.9979	0.263	3.943	0.21	18.72	0.9316	10.26	4.106	12.40
1.221	0.9946	0.714	6.198	0.48	21.95	0.9217	11.91	3.135	16.73
3.162	0.9866	1.835	8.313	1.24	25.00	0.9127	13.42	2.329	22.15
5.475	0.9766	3.149	8.837	2.27	27.78	0.9047	14.79	1.764	28.03
7.626	0.9694	4.349	8.520	3.38	30.38	0.8974	16.03	1.343	34.73
9.705	0.9616	5.491	7.795	4.60	33.92	0.8880	17.72	0.914	46.27
11.68	0.9546	6.560	7.105	5.92	36.12	0.8821	18.74	0.727	54.27
At 18° C. Weight of water used = 21.110 gm.									
0.397	0.9984	0.233	4.500	0.21	17.24	0.9328	9.461	5.837	15.61
1.168	0.9937	0.683	7.382	0.70	20.55	0.9222	11.15	4.407	21.40
3.109	0.9851	1.801	10.18	1.81	23.54	0.9126	12.64	3.376	27.93
5.421	0.9759	3.113	10.90	3.35	27.69	0.9000	14.66	2.261	39.51
7.632	0.9675	4.343	10.51	4.96	31.42	0.8890	16.44	1.529	53.17
9.729	0.9594	5.492	9.641	6.72	34.73	0.8794	17.97	1.069	68.73
13.61	0.9450	7.565	7.643	10.82					
At 25° C. Weight of water used = 21.016 gm.									
0.404	0.9971	0.237	5.290	0.31	11.51	0.9504	6.436	10.35	11.74
1.042	0.9931	0.608	8.207	0.84	15.22	0.9370	8.389	8.100	17.60
3.732	0.9810	2.154	11.76	2.38	18.61	0.9250	10.13	6.254	24.56
5.259	0.9749	3.016	12.75	4.43	23.20	0.9100	12.42	4.193	36.76
7.456	0.9660	4.238	12.36	6.66	27.30	0.8974	14.42	2.857	51.63
9.517	0.9581	5.364	11.42	9.09	30.97	0.8864	16.15	1.965	68.67

were used. The densities given in the above table are those due to Nichols and Wheeler (36). The column C_{NH_3} represents the concentration of ammonia in gram-mols per litre.

As in previous sections the regularity of the data, as exhibited by the plots, is a favorable point. Both the vapor pressure and specific conductivity curves lie in legitimate regions with respect to the different temperatures at which they were obtained.

The best vapor pressure data available with which to compare the present work are those given by Perman (41, p. 1397). Table XIV contains the

comparison of the data. At the higher concentrations Perman's data are slightly higher than those obtained in this work. A higher vapor pressure would indicate either the presence of some volatile impurity in the ammonia, a slight difference in temperature, or incomplete equilibrium. A temperature difference of 0.2° C. would account for this.

TABLE XIV

PARTIAL PRESSURES OF AMMONIA SOLUTIONS AT 10° C.

NH_3, %	4.16	8.26	12.32	15.88	20.54	21.83
Partial pressure of NH_3						
Perman (41)	1.65	3.72	6.42	9.51	14.92	16.98
Present work	1.65	3.72	6.30	9.20	14.50	16.40

On account of the great solubility of ammonia in water the vapor pressures are much lower than those of the two systems dealt with previously. Another reason for such low vapor pressures will be discussed later.

Conductivity data for ammonia are plentiful for dilute solutions but rather scarce for solutions of concentrations greater than 0.1 *N*. Considerable deviation is found in the low concentration data. The chief investigators have been Noyes and Kato (38) and Lunden (29, 30). It has been possible to overlap their range of concentration at only two points in the present work and the agreement there is not good. However, great accuracy is not claimed at the extremely low concentrations in this work since a slight amount of the ammonia injected into the cell at the beginning of a run might be absorbed by the stopcock grease and adsorbed on the glass walls of the system. This amount, though slight, would have a relatively large effect on the first value. The values obtained by Kohlrausch (25, p. 1078) up to concentrations of 30.5% check very well with the present data. Comparisons of the above-mentioned data are given in the following tables.

TABLE XV

SPECIFIC CONDUCTIVITY OF AMMONIA SOLUTIONS $\times 10^4$ (LOW CONCENTRATIONS)

Temp., °C.	C_{NH_3}	Lunden	Noyes and Kato	Present work
18	0.083	—	2.81	2.66
	0.10	3.18	3.10	—
25	0.10	3.70	3.62	3.41

It will be noted from the last column of Table XVI, that the Kohlrausch conductivity curve intersects that of the present work in two places but the difference is never very large. The average mean deviation over the whole range is 0.03 mhos.

TABLE XVI

SPECIFIC CONDUCTIVITY OF AMMONIA SOLUTIONS $\times 10^4$ (HIGH CONCENTRATIONS)

% NH_3	Kohlrausch	Present work	Difference	% NH_3	Kohlrausch	Present work	Difference
0.1	2.51	2.51	—	4.01	10.95	10.76	0.19
0.4	4.92	4.92	—	8.03	10.38	10.48	−0.10
0.8	6.57	6.57	—	16.15	6.32	6.46	−0.14
1.6	8.67	8.52	0.15	30.50	1.93	1.79	0.14

The conditions existing in aqueous solutions of ammonia may be expressed in two equations similar to (10) and (11) for sulphur dioxide.

$$C_{H_2O} = [H_2O] + [NH_4OH] + [OH^-] \tag{32}$$

$$C_{NH_3} = [NH_3] + [NH_4OH] + [OH^-] \tag{33}$$

By following a procedure similar to that used in the previous section an equation of the form of (16) is obtained.

$$a = Hp\,(1 + \frac{b + Hp}{K_1}) \tag{34}$$

In this case $a = C_{NH_3} - [OH^-]$ and $b = C_{H_2O} - C_{NH_3}$. On solving (34) for H and K_1, H was found to be extremely small and in some cases negative. In every case it was extremely small compared with the corresponding value of H for sulphur dioxide. This showed that the amount of uncombined ammonia in the liquid phase was negligible. It was borne out by the fact that the vapor pressures were relatively low as compared with those of solutions of sulphur dioxide where there is a considerable amount of uncombined reactant present.

Assuming that the amount of uncombined ammonia was negligible it was necessary to rearrange equation (34) to fit the case, if possible, and to prove that the above assumption was correct. Since H is small, Hp in the term $(b + Hp)$ is negligible compared with b. K_1 is also very small and the value of $\frac{b}{K_1}$ is very large compared with 1. Hence equation (34) becomes

$$a = \frac{Hpb}{K_1}$$

or

$$\frac{a}{b} = \frac{Hp}{K_1} = kp \tag{35}$$

which is the same as

$$\frac{C_{NH_3} - [OH^-]}{C_{H_2O} - C_{NH_3}} = kp \tag{36}$$

Since the value of OH^- is very small (36) may be written

$$\frac{C_{NH_3}}{C_{H_2O} - C_{NH_3}} = kp \tag{37}$$

without affecting the accuracy appreciably.

When $\frac{a}{b}$ was plotted against the partial vapor pressure of the ammonia at each temperature investigated straight lines were obtained as predicted by equation (35). From equation (37) the conclusion may then be drawn that the ratio of the concentration of the ammonia to that of the uncombined water is a constant function of the vapor pressure. It is of course impossible to differentiate between H and K_1 since the constant k gives the ratio of the two. But since a straight line relationship holds it follows that the value for K_1 must be very small, which means that by far the greatest part of the ammonia is combined. Since there is so little free ammonia in the solution the partial vapor pressure will be small in contrast with the two previous systems.

The conclusion drawn above is at variance with the results of Moore (34). Moore, using the partition coefficient of ammonia between water and chloroform, heats of neutralization, and heats of solution, calculated ionization constants for ammonia solutions and from the data thus obtained arrived at the conclusion that at 20° C. the ratio $\frac{NH_3}{NH_4OH}$ was approximately 2. The assumptions made by Moore are not justified and it was pointed out that they were at variance with the partial vapor pressures as registered by ammonia solutions.

Table XVII contains the theoretical data calculated from the experimental data contained in Table XIII. C_{H_2O} represents the water concentration in gram-mols per litre. $[OH^-]$ is the concentration of the OH^- (or NH_4^+) ion, also in gram-mols per litre. The values of a, b, and $\frac{a}{b}$ have been explained already. K_a is the true dissociation constant calculated in a similar manner

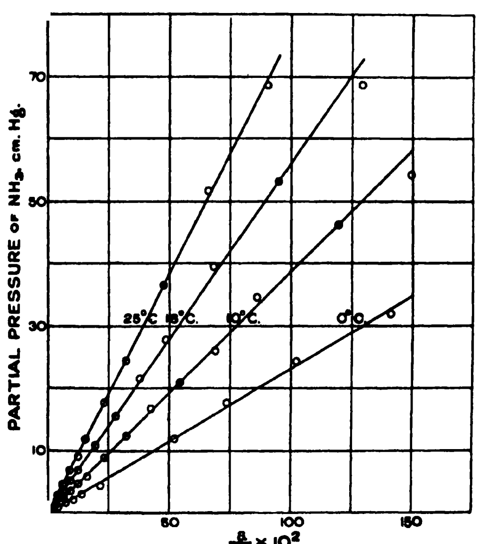


FIG. 12. *Relation of $\frac{a}{b}$ to partial vapor pressure of ammonia.*

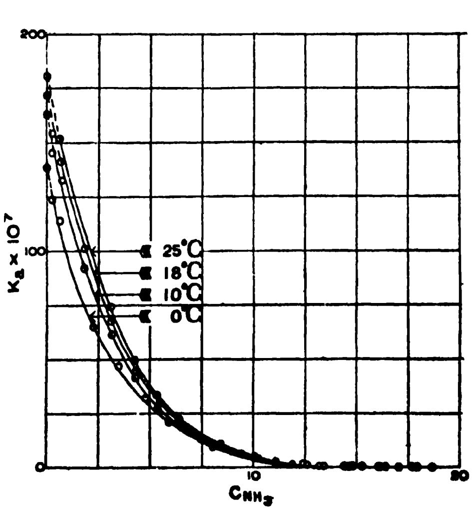


FIG. 13. *Relation of K_a to C_{NH_3} in the system ammonia-water.*

TABLE XVII

THEORETICAL DATA FOR THE SYSTEM AMMONIA-WATER CALCULATED FROM THE EXPERIMENTAL DATA IN TABLE XIII

C_{H_2O}	$[OH^-] \times 10^4$	a	b	$\frac{a}{b} \times 10^2$	$K_a \times 10^7$	C_{H_2O}	$[OH^-] \times 10^4$	a	b	$\frac{a}{b} \times 10^2$	$K_a \times 10^7$
At 0° C.											
55.17	11.29	0.244	54.92	0.445	124.6	45.00	27.74	8.092	36.91	21.93	9.51
54.67	26.71	0.625	54.04	1.157	114.1	41.53	17.89	10.84	30.69	35.33	2.95
52.53	38.41	2.245	50.28	4.465	65.70	38.55	11.24	13.20	25.35	52.07	0.95
50.78	41.16	3.579	47.20	7.580	47.34	35.98	7.29	15.26	20.72	73.64	0.35
49.18	39.44	4.823	44.35	10.87	32.25	33.76	4.75	17.06	16.70	102.1	0.13
47.69	35.80	5.967	41.72	14.30	21.47	31.81	3.13	18.64	13.17	141.6	0.05
At 10° C.											
55.33	13.55	0.124	55.21	0.22	148.2	44.29	26.66	8.508	35.78	23.78	8.35
55.13	19.56	0.261	54.87	0.47	146.4	42.04	20.37	10.26	31.78	32.27	4.04
54.54	30.75	0.711	53.83	1.32	132.9	39.93	15.55	11.91	28.02	42.50	2.03
53.04	41.25	1.831	51.21	3.57	92.92	37.99	11.55	13.42	24.57	54.62	0.99
51.29	43.84	3.144	48.14	6.53	61.13	36.25	8.75	14.79	21.46	68.93	0.51
49.69	42.27	4.344	45.34	9.58	41.12	34.67	6.66	16.03	18.64	86.00	0.28
48.19	38.68	5.487	42.70	12.85	27.26	32.56	4.53	17.72	14.84	119.00	0.12
46.79	35.26	6.566	40.23	16.29	18.95	31.27	3.61	18.74	12.53	149.60	0.07
At 18° C.											
55.20	18.91	0.231	54.97	0.42	154.6	42.85	24.52	9.459	33.39	28.32	6.36
54.50	31.01	0.679	53.82	1.26	141.5	40.66	18.52	11.14	29.51	37.79	3.07
52.97	42.76	1.796	51.17	3.51	101.7	38.73	14.19	12.64	26.09	48.45	1.59
51.23	45.80	3.108	48.12	6.46	67.47	36.12	9.49	14.66	21.46	68.32	0.61
49.60	44.16	4.339	45.26	9.59	44.93	33.84	6.42	16.44	17.40	94.50	0.25
48.06	40.51	5.488	42.57	12.89	29.89	31.86	4.49	17.97	13.89	129.30	0.11
45.31	32.11	7.562	37.75	20.03	13.64						
At 25° C.											
55.11	19.61	0.235	54.87	0.428	163.4	46.68	38.35	6.432	40.24	15.99	22.88
54.53	30.42	0.605	53.92	1.123	152.8	44.09	30.02	8.386	35.70	23.48	10.75
52.42	43.59	2.149	50.27	4.275	88.43	41.78	23.18	10.13	31.65	32.00	5.30
51.26	47.25	3.011	48.24	6.241	74.15	38.79	15.53	12.42	26.37	47.10	1.94
49.62	45.81	4.233	45.38	9.326	49.57	36.21	10.59	14.42	21.79	66.18	0.77
48.11	42.33	5.360	42.75	12.54	33.42	33.95	7.28	16.15	17.80	90.72	0.33

TABLE XVIII

IONIC MOBILITIES OF AQUEOUS AMMONIA (KOHLRAUSCH)

Temp., ° C.	NH_4^+	OH^-	NH_4OH	Temp., °C.	NH_4^+	OH^-	NH_4OH
0	38.43	117.6	156.0	18	64.00	174.0	238.0
10	52.64	148.9	201.5	25	73.92	195.9	269.8

to that used for the apparent dissociation constant for sulphur dioxide and carbon dioxide. In this case it is the true dissociation constant on account of the complete combination of the ammonia with water, the conductivity thus giving a true measure of the fraction of NH_4OH which is ionized.

The ionic mobilities were calculated from the data of Kohlrausch (26) and are given in Table XVIII.

Fig. 12 shows the straight line relationships obtained by plotting partial pressure of ammonia against the values of $\frac{a}{b}$. Fig. 13 indicates the variation of K_a with rising concentration. In Fig. 12 it will be noted that there is a slight wave in the points before and after the value of the concentration where $[OH^-]$ reaches a maximum. This would be expected from equation (36) but the deviation from a straight line is so small that the assumption of almost complete combination of ammonia with water is not invalidated. The values of the constant k are given in Table XIX.

TABLE XIX

VALUES OF CONSTANT k

Temp., °C.	0	10	18	25
k	0.0431	0.0258	0.0178	0.0130

The decrease of k with rise in temperature is to be expected since $k = \frac{H}{K_1}$, since H is bound to decrease and K_1 to increase with rise in temperature. An investigation at high temperatures would therefore be of interest since in that region $\frac{b}{K_1}$ will no longer be extremely large compared to 1.

In Fig. 13 the dotted portions of the curves are the extrapolations to the values given in the literature for very dilute solutions. It will be noted that these extrapolations continue quite regularly from the maximum values of K_a obtained in the present work. A further discussion of K_a will be included in the following section.

Discussion

In the derivations that have been made with regard to existing equilibria the existence of certain assumptions has been indicated and it may be well to emphasize once more that the authors are aware of the approximate nature of their calculations. For instance, there is no doubt that the use of the limiting conductivities used at high concentrations cannot lead to exact results. In the light of existing data it was impossible to do otherwise and that the calculations are warranted is brought out by the agreement between the experimental results and the formulas which were derived. In the following paragraphs further evidence will be offered that these speculations are justified in giving a general insight into the equilibria existing in the systems investigated.

It is evident that in the three systems there is a distinct variation of properties. Carbon dioxide is relatively insoluble, and in solution is ionized only to a slight extent. Sulphur dioxide is more soluble and in solution it is more highly ionized, while ammonia is extremely soluble, slightly ionized, and basic. Due to the wide variation of the properties of these systems, marked differences in the types of equilibria existing would be expected. This has been found to be the case.

Vapor pressure data show that ammonia has the greatest affinity for water, sulphur dioxide is next, and carbon dioxide has the smallest of the three. For this reason it was impossible to reach sufficiently high concentrations of carbon dioxide, with the means at hand, to arrive at a true measure of the equilibrium. Some advance has been made but it will be necessary to proceed to higher concentrations in order to subject it to the type of theoretical treatment used in the cases of sulphur dioxide and ammonia where the change in the water concentration plays an important part.

The relative magnitudes of the affinity of each of these gases for water may be deduced. By multiplying the mol fraction of the gas present in the solutions investigated by the vapor pressure of the pure gas in the liquid state at the same temperature it is possible to calculate the vapor pressure if an ideal solution results. Table XX shows the vapor pressures obtained from this calculation compared with the experimental pressures. C_{gas} is the concentration in gram-mols per litre and the pressures are given in centimetres of mercury.

TABLE XX

COMPARISON OF EXPERIMENTAL AND CALCULATED VAPOR PRESSURES

Gas	C_{gas}	$P_{calc.}$	$P_{meas.}$	$\frac{P_{meas.}}{P_{calc.}}$
CO_2	0.0835	3.93	85.30	21.70
SO_2	0.1393	0.29	1.97	6.79
NH_3	0.2460	1.44	0.15	0.10

It will be noted that $P_{meas.}$ is much greater than $P_{calc.}$ for carbon dioxide and sulphur dioxide and much less for ammonia. This is in good agreement with the data since carbon dioxide was found to be least combined of the three, a smaller amount of uncombined gas was present in the sulphur dioxide solutions, while ammonia, to an approximation, was largely combined.

It should be stated here that the partial vapor pressure above solutions of ammonia is due to NH_3 and not to NH_4OH as might be expected. It has been shown by Carpenter (9) that the amount of combination between ammonia and water in the vapor phase is much below 1%.

On reviewing previous tables it will be seen that the values of K_a, the apparent dissociation constant, decrease with rising temperature in the case of sulphur dioxide and increase in the cases of carbon dioxide and ammonia. It

will be remembered from a previous section that the constants K_a, K_1, and K_2 are connected by the relation

$$K_a = \frac{K_2[H_2O]}{K_1+[H_2O]}$$

$$= K_2 \frac{1}{1 + \frac{K_1}{[H_2O]}} \quad (38)$$

The above equation holds in the case of sulphur dioxide where $\frac{K_1}{[H_2O]}$ was shown to have the same order of magnitude as 1. In the case of ammonia where $\frac{K}{[H_2O]}$ is much less than 1, $K_a = K_2$, *i.e.*, the true and apparent dissociation constants are identical. In the case of carbon dioxide, on the other hand, since $\frac{K_1}{[H_2O]}$ is much greater than 1, the equation becomes $K_a = \frac{K_2}{K_1}[H_2O]$, and it is impossible to estimate the true dissociation constant since K_1 could not be determined from the data.

Some light is thrown on the temperature variation of the apparent dissociation constant by the above considerations. From the heat of neutralization data in the literature (27, pp. 1547-1548) the following thermal equations can be derived as holding between 18 and 25° C.

$$NH_4^+ + OH^- = NH_4OH + 1500 \text{ cal.}$$

$$H^+ + HCO_3^- = H_2CO_3 + 3700 \text{ cal.}$$

$$H^+ + HSO_3^- = H_2SO_3 - 2200 \text{ cal.}$$

It follows that the true dissociation constant will increase with rise in temperature for the ammonia and carbon dioxide systems and decrease for the sulphurous acid system. Applying the Van't Hoff isochore to calculate the percentage increase between 18 and 25° C. an increase of 6% is predicted in the case of ammonia. This compares favorably with the 5% increase of the apparent dissociation constant of ammonia, which as stated above, should be the same as the true dissociation constant.

In the case of sulphur dioxide the true dissociation constant is given by the equation

$$K_2 = \frac{[HSO_3^-]}{C_{SO_2} - Hp - [HSO_3^-]}$$

The following table gives these values calculated for various concentrations at 25 and 18° C.

Taking the average as 0.0325 at 25° C. and 0.0348 at 18° C. there is a decrease of between 7 and 8% which agrees favorably with 9% decrease calculated from the thermal data. The apparent dissociation constant decreases by 16% but this is due to the increase in K_1 with rise of temperature. Equation 38 predicts just this result from the values of K_1 for sulphur dioxide.

TABLE XXI

VALUES OF THE TRUE DISSOCIATION CONSTANTS FOR SULPHUR DIOXIDE SOLUTIONS

18° C.				25° C.			
SO_2 %	K_2	SO_2 %	K_2	SO_2 %	K_2	SO_2 %	K_2
1.103	0.03339	7.340	0.03511	1.809	0.03148	7.640	0.03261
2.458	0.03485	8.831	0.03458	3.116	0.03280	8.756	0.03183
4.150	0.03502	10.30	0.03402	4.672	0.03321		
5.773	0.03531			6.184	0.03310		

**Values of K_2 below 1% sulphur dioxide cannot be determined accurately and hence are not included.*

Consider now carbon dioxide. Where the thermal data predict an increase of 16% in the true dissociation constant, K_2, over the seven degree range, the actual measurements of the apparent dissociation constant show only a 10% increase. However this is to be expected, since K_a is proportional to $\frac{K_2}{K_1}$ and K_1 must increase with rise in temperature and evidently does so to the extent of some 6% over this range.

The above discussion of the variation of the apparent dissociation constant with the temperature is of considerable interest because of the evidence it affords for the following generalizations.

(1) Ammonia, when dissolved in water at ordinary temperatures, is almost completely combined so that the apparent and true dissociation constants are the same.

(2) Sulphur dioxide, in aqueous solution exists partly in the combined state, partly uncombined, to an extent which can be calculated. With rise in temperature the amount of uncombined sulphur dioxide increases rapidly. The true dissociation constant can be calculated approximately.

(3) Carbon dioxide, at ordinary temperatures and pressures, is only slightly combined in its aqueous solutions and becomes decreasingly so with rising temperature. More data at high pressures are necessary to make any estimate of the true dissociation constant.

References

1. BALY, E. C. C. and BAILEY, R. A. J. Chem. Soc. 121: 1813-1821. 1922.
2. BARTH, K. Z. physik. Chem. 9: 176-219. 1892.
3. BAUD, E. and GAY, L. Ann. chim. phys. 17: 398-418. 1909.
4. BERTHELOT, M. Ann. chim. phys. (6) 1: 173. 1884.
5. BOHR, C. Ann. phys. 68: 500-525. 1899.
6. BUCH, K. Soc. Sci. Fennica Commentationes Phys. Math. 2, No. 16, 1-9. 1925.
7. BURKE, W. M. J. Am. Chem. Soc. 42: 2500-2506. 1920.
8. CAMPBELL, W. B. and MAASS, O. Can. J. Research, 2: 42-64. 1930.
9. CARPENTER, G. B. Unpublished thesis, McGill University. 1928.
10. DRUCKER, K. Z. physik. Chem. 49: 563-589. 1904.
11. ENCKELL, J. Papierfabr. 23: 633-636. 1925.
12. FINDLAY, A. and CREIGHTON, H. J. M. J. Chem. Soc. 97: 536-561. 1910.

13. FINDLAY, A. and HOWELL, O. R. J. Chem. Soc. 107: 282-284. 1915.
14. FINDLAY, A. and SHEN, B. J. Chem. Soc. 101: 1459-1468. 1912.
15. FINDLAY, A. and WILLIAMS, T. J. Chem. Soc. 103: 636-645. 1913.
16. FOOTE, H. W. J. Am. Chem. Soc. 43: 1031-1038. 1921.
17. FULDA, W. Arb. Kais. Gesund.-Amt. 30: 81. 1909.
18. HOUSEKEEPER, W. G. J. Am. Inst. Elect. Eng. 42: 954-960. 1923.
19. HUDSON, J. C. J. Chem. Soc. 127: 1332-1347. 1925.
20. JUST, G. Z. physik. Chem. 37: 342-367. 1901.
21. KANOLT, C. W. J. Am. Chem. Soc. 29: 1402-1416. 1907.
22. KENDALL, J. J. Am. Chem. Soc. 38: 1480-1497. 1916.
23. KERP, W. and BAUR, E. Arb. Kais. Gesund.-Amt. 26: 297-300. 1907.
24. KNOX. Ann. phys. 55: 44. 1895.
25. KOHLRAUSCH, F. Landolt and Börnstein, Physikalische-Chemische Tabellen, Vol. 2, 1923.
26. KOHLRAUSCH, F. and HOLBORN, L. Das Leitvermogen der Elektrolyte. 1898.
27. LANDOLT and BORNSTEIN. Physickalische-Chemische Tabellen, Vol. 2. 1923.
28. LINDNER, J. Monatsh. 33: 613-672. 1912.
29. LUNDEN, H. J. chim. phys. 5: 145-185. 1907.
30. LUNDEN, H. J. chim. phys. 5: 574-608. 1907.
31. MAASS, C. Unpublished thesis, McGill University.
32. MAASS, C. E. and MAASS, O. J. Am. Chem. Soc. 50: 1352-1368. 1928.
33. MCRAE, J. and WILSON, W. E. Z. anorg. Chem. 35: 11. 1903.
34. MOORE, T. S. J. Chem. Soc. 91: 1379-1384. 1907.
35. NEUHAUSEN, B. S. and PATRICK, W. A. J. Phys. Chem. 25: 693-720. 1922.
36. NICHOLS and WHEELER. International Critical Tables, Vol. 3. 1926.
37. NOYES, A. A. and coworkers. Landolt and Börnstein, Physikalische-Chemische Tabellen, 5th. ed. Erster Erganzungband. 1927.
38. NOYES, A. A. and KATO, Y. J. Am. Chem. Soc. 30: 335-353. 1908.
39. OMAN, E. Tekn. Tidsckrift. Kemi. 54: 81. 1924.
40. OSTWALD, W. J. prakt. Chem. (2) 32: 314. 1885.
41. PERMAN, E. P., Landolt and Börnstein, Physikalische-Chemische Tabellen, Vol. 2. 1923.
42. PFEIFFER, E. Ann. Phys. 23: 625-650. 1884.
43. SANDER, W. Z. phys. Chem. 78: 513-549. 1912.
44. SIMS. J. Chem. Soc. 14: 1. 1862.
45. SMITH, W. T. and PARKHURST, R. B. J. Am. Chem Soc. 44: 1918-1927. 1922.
46. THOMSEN, J. Thermochem. Unters. 1:169. 1882.
47. WALDEN, P. and CENTNERSZWER, M. Z. physik. Chem. 42: 432-468. 1903.
48. WALKER, J. J. Chem. Soc. 77: 1110-1114. 1900.
49. WALKER, J. and CORMACK, W. J. Chem. Soc. 77: 5-21. 1900.
50. WILKE, E. Z. anorg. allgem. Chem. 119: 365-379. 1922.
51. WRIGHT, R. J. Chem. Soc. 105: 2907-2909. 1914.

THE EFFECT OF TEMPERATURE ON THE EXPRESSION OF FACTORS GOVERNING RUST REACTION IN A CROSS BETWEEN TWO VARIETIES OF *TRITICUM VULGARE*[1]

BY J. B. HARRINGTON[2]

Abstract

Two random populations of F_2 plants of the cross Marquillo x Marquis were tested for the reaction of their F_3 seedling progenies to form 21 of *Puccinia graminis tritici* in the greenhouse, at average daily temperatures of 69.7° F. (the warm test) for one population, and 60.6° F. (the cool test) for the other. In both tests Marquis was susceptible and Marquillo was resistant. In the "warm test" ten families of a total of 781 were resistant. In the "cool test" five families of a total of 301 were susceptible. In both cases the results fitted a 63:1 ratio excellently, indicating the operation of three main genetic factors for rust reaction. A genetic hypothesis is proposed that explains the results on the basis of the influence of low temperature in curtailing the action of three susceptibility factors A, B and C carried by Marquis. The results indicate that genetic studies on characters which are easily influenced by environmental conditions should be made under controlled conditions, after ascertaining in advance the general effects of different temperatures, etc., upon the hybrid material to be used.

One of the greatest difficulties in conducting genetic studies on rust reaction has been the influence of environmental conditions, especially temperature and light, upon rust development. Of these two the influence of temperature appears to be of more practical importance, since, with a reasonable duration and intensity of light, rust develops more or less fully. The pronounced influence exerted by temperature on the development of cereal rusts has been pointed out by various investigators, including Peltier (3), Stakman and Levine (6), and quite recently by Gordon (1), Peturson (4) and Johnson (2). The work of these last three investigators is particularly interesting to the geneticist and plant breeder, since it offers at least a partial explanation of many baffling results that have been obtained.

Gordon (1) found that Joanette strain of oats showed a striking difference in its reactions to physiologic forms 1, 3, 4 and 5 of *Puccinia graminis avenae* at a low (57.4° F.) and at a high (75.4° F.) temperature. At the low temperature Joanette strain was resistant to forms 1, 3 and 4, and heterozygous to form 5, but at the high temperature it was heterozygous to forms 1 and 3 and susceptible to forms 4 and 5.

Peturson (4), working with *Puccinia coronata avenae*, obtained equally interesting results. Green Mountain and two other oat varieties were highly resistant to form 7 at 57° F., but fully susceptible at 77° F. Yet the same

[1] *Manuscript received May 7, 1931.*

Contribution from the laboratories of the University of Saskatchewan, Canada, with financial assistance from the National Research Council of Canada. This study forms a part of a co-operative attack on the problem of cereal rust in Canada, carried on jointly by the National Research Council, the Federal Department of Agriculture and the Universities of Manitoba, Saskatchewan and Alberta. The results were reported in full at the meeting of the Associate Committee on Field Crop Diseases at Winnipeg, April 10, 1931.

[2] *Professor of Field Husbandry, University of Saskatchewan.*

varieties were quite susceptible to form 4 at both temperatures. Other varieties were susceptible to both forms at both temperatures.

Results obtained by Johnson (2) with *Puccinia graminis tritici* also demonstrated that physiologic forms do not behave identically with respect to temperature. Johnson showed that Mindum and two other durum wheats which give at average greenhouse temperatures an X type reaction to ten different forms of rust were more or less resistant at low temperatures and more or less susceptible at high temperatures. For Mindum the temperature range necessary to give the difference between resistance and susceptibility varied from 7° to 13° F. for the different forms. He also found that when these varieties were infected by physiologic forms, to which they are normally resistant, there was practically no difference observable in the rust reactions obtained at the two different temperatures. Similarly, in the case of forms to which these wheats are normally susceptible, there was no difference to be seen in the ultimate rust reactions at the high and low temperatures.

The plant breeder works largely with hybrid material and he wonders, in connection with this last piece of work, what the reaction of hybrids would be when the parent varieties were (a) of the X reaction group, and (b) of the group that reacted the same whether the temperature was high or low. A striking example in answer to the last question was furnished by the results obtained from a hybrid population used in the rust research breeding work at the University of Saskatchewan.

Experimental Procedure

A random population of 781 F_2 plants of the cross Marquillo x Marquis was tested by means of F_3 seedling progeny to physiologic form 21 of *Puccinia graminis tritici* in the greenhouse during October and November 1926, at a temperature that was kept as close to 70° F. as was possible without the use of automatic controls. The average temperature during the period from the first inoculation, October 17, until seven days after the last inoculation, November 9, was 69.7° F. For convenience this test will be referred to as the "warm test". The average daily hours of sunshine was 4.0 and the average daily outdoor temperature was 32.7° F., the minimum being 12.6° F. and the maximum 49.3° F. The outside temperatures were not low enough at any time to cause a coating of ice or snow to remain on the greenhouse glass. The temperature and sunshine data are shown graphically in Fig. 1.

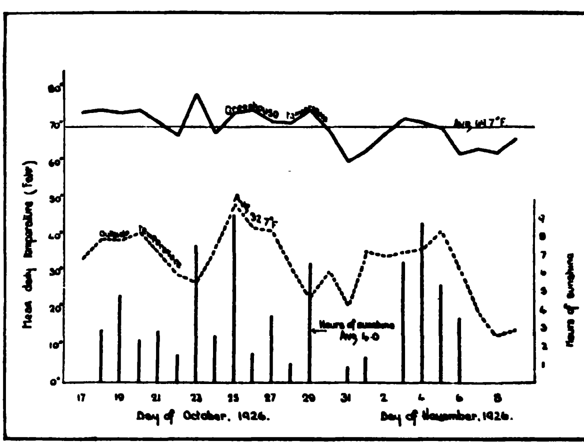


FIG. 1. *Greenhouse and outside temperature and hours of sunshine for the "warm test".*

A further test of 301 F_3 families from the same random lot of F_2 material (kept in reserve since 1926) was made during December 1930 in the same greenhouse with conditions as nearly as possible similar to those of 1926, excepting that the temperature was kept close to 61° F., the average temperature being 60.6° F. for the period of test, December 2 to 15. For convenience this test will be called the "cool test". The average daily hours of sunshine was 3.6; the average daily outdoor temperature was 23.8° F. with the minimum at 6.3° F. and the maximum at 30.6° F. As in the 1926 test, the outside temperatures were not low enough to cause a coating of ice on the greenhouse glass. The hours of sunshine averaged almost the same in the two tests, but as the sun in December gives a lower light intensity than in October or early November, supplementary artificial light was furnished in 1930 from 5 p.m. to 11:30 p.m. each night, by means of 200-watt Mazda lamps, each illuminating one square yard of greenhouse bench. It is considered by the writer, from frequent inspection of the infected seedlings in each test, that the general light environment in the 1926 test was fairly comparable to that of the 1930 test. The 1930 temperature and sunshine data are given in Fig. 2.

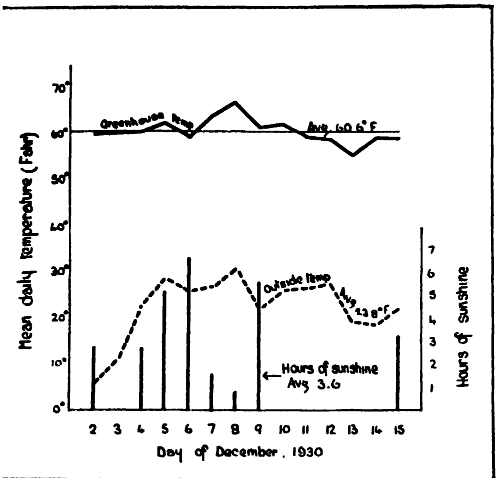


FIG. 2. *Greenhouse and outside temperature and hours of sunshine for the "cool test".*

Results

The results obtained from the two tests are given in Tables I and II. It is quite clear from Table I that susceptibility was dominant in the "warm test". Only ten families of a total of 781 were resistant. It is similarly clear from Table II that resistance was dominant in the "cool test". Five families were susceptible in a total of 301. The parent varieties, however, gave practically the same results regardless of the temperature, Marquis being susceptible in both tests and Marquillo resistant in both. These varietal behaviors are in full agreement with those obtained by Johnson (2) working with Mindum and Speltz Marz.

Perhaps the simplest and most satisfactory genetic analysis of the data consists in treating each set of results from the point of view of the fully recessive class as compared with all other classes. This is done in Table III. The X^2 test for the "warm test" results show an excellent fit to a 63:1 ratio ($P = 0.73$). If the susceptible F_2 plants (about 50) which were discarded in the field were included, the fit should still be very good ($P = 0.59$). The "cool

test" results showed an exceedingly good fit to a 63:1 ratio (P = 0.89). Thus, although the two sets of results are almost complete opposites from the point of view of resistance and susceptibility, they agree in indicating the operation of three main genetic factors for rust reaction.

TABLE I

DISTRIBUTION OF 781 F_2 PLANTS* OF THE CROSS MARQUILLO x MARQUIS, ACCORDING TO THE REACTION OF THEIR F_3 SEEDLING PROGENY TO FORM 21 IN THE GREENHOUSE AT A TEMPERATURE OF APPROXIMATELY 70° F. (WARM TEST)

Material	Distribution of F_2 plants according to the classes determined by the reaction of F_2 seedlings					Number of F_3 families
	R**	HR	H and I	HS	S	
Marquis					15	
Marquillo	13					
F_2	11	29	91	261	389	781

**This was not a complete random sample with respect to rust reaction, since approximately 50 of the most susceptible plants were discarded in the field.*

***R, resistant; HR heterozygous resistant; H, heterozygous; I, intermediate; HS, heterozygous susceptible; S, susceptible.*

TABLE II

DISTRIBUTION OF 301 F_2 PLANTS OF THE CROSS MARQUILLO x MARQUIS, ACCORDING TO THE REACTION OF THEIR F_3 SEEDLING PROGENY TO FORM 21 IN THE GREENHOUSE AT A TEMPERATURE OF APPROXIMATELY 61° F. (COOL TEST)

Material	Distribution of F_2 plants according to the classes determined by the reaction of F_3 seedlings					Number of F_3 families
	R*	HR	H and I	HS	S	
Marquis					20	
Marquillo	18					
F_2	209	68	14	5	5	301

**See footnote of Table I.*

TABLE III

COMPARISON OF THE RESULTS OF THE TWO TESTS AND THE PROBABLE FIT TO A 63:1 GENETIC RATIO IN EACH CASE

Test	Distribution of F_2 plants according to the F_3 reactions			X^2	P	Fit to a 63:1 ratio
	R	H	S			
"Warm test"	11		770*	0.12	0.73	Excellent
"Cool test"		296	5	0.02	0.89	Excellent

**The addition of the very susceptible plants discarded in the field would augment this number by approximately 50, making $X^2 = 0.31$ and $P = 0.59$, which still shows an excellent fit.*

Discussion of Results

The extreme difference in the results obtained from practically identical F_2 populations tested at different temperatures in the greenhouse to the same form of rust is of both genetic and economic interest. The F_2 populations are reasonably large in each case and arose from seed taken from the same bag, the population for the "warm test" being grown in 1926 and the population for the "cool test" in 1930. Both populations were normal in growth and similar in the character "plant height" for which they were studied. The F_3 families of each population were tested in the same greenhouse with the same equipment, and all environmental factors that were under the control of the technician who looked after the material were kept as nearly as possible the same, with the exception of temperature. The temperature in 1926 averaged 69.7° F., whereas in 1930 it averaged 60.6° F.

The amount and intensity of light in the two tests were not the same, owing to the tests being made in different years and at slightly different times of year. However, it does not seem probable that the light differences were responsible to any large extent for the great differences in rust reaction that occurred. The writer has frequently found that moderate differences in light intensity and duration did not cause large differences in the reactions of varieties or hybrids in seedling tests.

There were some distinct variations in the average daily greenhouse temperatures in the 1926 test due to unavoidable circumstances. Since the average difference of 9.1° between the "warm test" and the "cool test" brought about such great differences in the reactions of F_3 families, it might be thought that the two day duration of approximately 65° between October 30 and November 1 in the "warm test" would detrimentally affect the hybrid seedling reactions. This influence, if any, should be revealed by a fluctuation characterized by an increase in the proportion of resistant families for that portion of the test. No such variation in the results was found, as the analysis in Table IV shows. This table gives the rust classes of the F_3 families in each test according to the incubation periods as summarized by weekly groups.

TABLE IV

DISTRIBUTION OF F_3 FAMILIES OF BOTH TESTS BY RUST CLASSES ACCORDING TO THE INOCULATION DATES AS SUMMARIZED INTO WEEKLY GROUPS

Inoculation dates	R	HR	H and I	HS	S
Oct. 17–23, 1926	3	4	10	39	52
Oct. 24–31, 1926	2	7	18	72	125
Nov. 1– 8, 1926	4	20	62	146	206
Dec. 2– 9, 1930	115	20	36	3	3
Dec. 7–14, 1930	94	8	18	2	2

Genetic Considerations

A genetic hypothesis to explain the difference between the two sets of results is proposed as follows: Considering that three main genetic factors

govern rust reaction in the cross Marquillo x Marquis, let these be called A, B and C. Then either Marquis or Marquillo may be considered to have the constitution AABBCC. As susceptibility was dominant in Marquillo x Marquis hybrids when grown in the field and was also dominant in the "warm test" in the greenhouse, the constitution AABBCC may logically be assigned to Marquis and aabbcc to Marquillo. Let A, B and C be factors which when all are present in the homozygous condition cause susceptibility whether the temperature is around 61° or around 70° F. Then in either test Marquis and one sixty-fourth of the F_3 families would appear equally susceptible. When these factors are completely absent no susceptibility develops, whether the temperature approximates either 61° or 70°. Then Marquillo and one sixty-fourth of the F_3 families would be resistant in both tests.

Let these factors be such that their action in causing susceptibility is greatly reduced or curtailed at moderately low temperatures as compared with their action at moderately high temperatures. Then, when one or more of them is present in single or double dose in a warm greenhouse test, more or less susceptibility is evident, depending upon the dosage and perhaps upon differential effects of the different factors. But when a test is made in a cool greenhouse, little susceptibility occurs unless four or five doses of A, B and C are present, and full susceptibility occurs only when each factor is present in double dose (homozygous) with a total of six doses concerned. In the "warm test" the complete heterozygote AaBbCc would be about as susceptible as any individual carrying four, five or six doses, viz: AaBBCC, but in the "cool test" the AaBbCc individuals would show very little susceptibility.

This is a fairly simple hypothesis, having as its chief feature the radical curtailment of the action of the susceptibility factors when the temperature is low.* The hypothesis carries the implication that one or two doses of A, B or C produces as much susceptibility at 70° F. as four or five doses would at 61° F. The hypothesis explains the results satisfactorily. This is brought out graphically in Fig. 3, where the rust class distribution for each

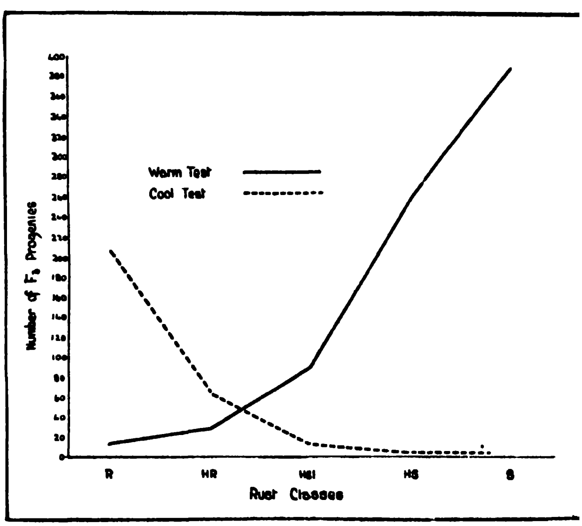


FIG. 3. *Distribution of Marquillo × Marquis F_2 plants according to the reaction of F_3 seedling progeny at different temperatures.*

**Whether the temperature exerts a direct effect on the parasite or on the host or on both, or whether complicated indirect actions are involved, is not known. For the time being the expression "curtailment of action of the susceptibility factors" seems to be appropriate.*

test is shown. The hypothesis is supported indirectly by both pathological and genetical findings in which lowered temperatures have been found to result in lowered pathogenic activity. The present experiment, however, is the first reported case, as far as the writer knows, where the influence of temperature appeared to be responsible for the reversal of the proportions in a genetic ratio.

A somewhat similar, though much less striking, case of the effect of different environments upon similar hybrid populations was reported recently by Quisenberry (5) in connection with the inheritance of winter hardiness. He had seed of the same group of F_3 families sown at St. Paul, Minn., and at Moccasin, Montana. At St. Paul the winter conditions were more severe than at Moccasin and approximately three times as many hybrid lines were tender than were hardy. At Moccasin the opposite was true.

The results of these studies clearly indicate that genetic studies on characters which are easily influenced by environmental conditions should be made under controlled conditions, after ascertaining in advance the general effects of different temperatures, light conditions, etc., upon the hybrid material to be used.

It is of interest to consider the effect of results such as those reported here upon the usual designations attached to factors. For example, should factors A, B and C be called dominant factors for susceptibility when they produce very little susceptibility at 61° F? Should they be called dominant for susceptibility when 209 of a total of 301 F_2 plants show (as in the cool test) as high resistance as Marquillo which possesses none of these factors? While such questions tend to place the terms "resistant" and "susceptible" in a rather precarious position, it is nevertheless true that these terms are generally used to refer to performance under ordinary field conditions. Perhaps confusion may be avoided by referring to such factors as "field susceptibility factors" rather than as "susceptibility factors".

There is one further genetical point that might be considered here. It is possible that in some crosses one or more of the genetic factors controlling rust reaction might be quite susceptible to temperature influences and the other controlling genetic factors be free from such influences. In such a case a genetic analysis, even with controlled conditions, might prove to be very difficult. It is easy to see why the disturbing factor just mentioned, together with the uncontrolled conditions in a field nursery, might make exact genetic analysis of field results an impossibility.

Breeding Considerations

The same F_3 families that were tested in the greenhouse in October and November 1926 were grown in the field nursery in 1927, where there was a uniformly heavy epidemic. The nursery results were closely like those obtained in the greenhouse from seedlings tested at approximately 70° F. to form 21. The use of form 21 in the greenhouse was particularly appropriate, since it is a prominent form in western Canada and appeared to be the most prevalent form in the nursery during the 1927 epidemic. In addition to

form 21, forms 15, 17, 29, 30 and 36 were found in this nursery.* The relationship for F_2 plants between the reaction of their F_3 seedling progeny to form 21, and the reaction of maturing F_3 progeny in the nursery to the mixture of forms including form 21, was determined by use of the contingency surface. The coefficient of contingency was 0.69 ± 0.062, indicating a strong positive relationship.

While no relationship between the "cool test" and field results has been obtained, it is apparent that there would be no positive correlation but probably a strong negative one if the field results were secured under conditions such as characterized the 1927 crop season. This point is extremely significant, because the "warm test" proved to be highly effective in the selection of resistant hybrids and made possible the reduction of 4,200 field harvested hybrid lines to only 419 during the winter months, whereas the "cool test" would have had a negative value in this regard, for it would have been deceiving.

The importance of this work to the practical breeder can hardly be overestimated. The present results suggest that wherever greenhouse, or indeed field nursery, results on reactions to pathogenes are to be used as deciding factors in the selection of hybrid plants or lines, all possible information should be obtained at a very early stage in the breeding project (preferably by means of a small experimental F_2 population) on the effects of different temperatures with respect to reactions to representative or leading physiologic forms of the pathogene used. This information would be the basis for making controlled tests under conditions that would promote the greatest effectiveness in making selections.

References

1. GORDON, W. L. Effect of temperature on host reactions to physiologic forms of *Puccinia graminis avenae*, Erikss. and Henn. Sci. Agr. 11: 95-103. 1930.
2. JOHNSON, T. A study of the effect of environmental factors on the variability of physiologic forms of *Puccinia graminis tritici*, Erikss. and Henn. Dom. Can. Dept. Agr. Bull. 140, n.s. 1931.
3. PELTIER, G. L. A study of the environmental conditions influencing the development of stem rust in the absence of an alternate host. II. Infection studies with *Puccinia graminis tritici*, Form III and Form IX. Nebraska Agr. Exptl. Sta. Research Bull. 25. 1923.
4. PETURSON, B. Effect of temperature on host reactions to physiologic forms of *Puccinia coronata avenae*. Sci. Agr. 11: 104-110. 1930.
5. QUISENBERRY, K. S. Inheritance of winter hardiness, growth habit and stem-rust reaction in crosses between Minhardi winter and H-44 spring wheats. U.S. Dept. Agr. Tech. Bull. 218. 1931.
6. STAKMAN, E. C. and LEVINE, M. N. Effect of certain ecological factors on the morphology of the Urediniospores of *Puccinia graminis*. J. Agr. Research, 16: 43-72. 1919.

**The writer is indebted to Doctors Newton and Johnson of the Dominion Rust Research Laboratory, Winnipeg, for identifying these cultures and for supplying pure cultures for greenhouse studies.*

THE RELATIONSHIP BETWEEN ENDOSPERM DEVELOPMENT AND MORPHOLOGIC CHARACTERS IN THE F_2 GENERATION OF A *T. DICOCCUM* X *T. VULGARE* CROSS[1]

BY J. B. HARRINGTON[2]

Abstract

The relationship between the degree of plumpness of F_2 seeds and the type of the resulting F_2 plants was studied in the cross Vernal (*T. dicoccum*) x Marquis (*T. vulgare*). A random sample of F_2 seeds was divided into three classes based upon kernel plumpness, *viz.* plump (Class A), slightly shrunken (Class B), and shrunken (Class C). In these there were by number 55.5, 41.0 and 3.5% of seeds respectively. Emergence of F_2 plants in the field was 64, 58 and 36% for Classes A, B and C. The F_2 plants were studied for 13 morphological characters. Comparing the populations for all characters combined, Class A was more *dicoccum*-like than Class B, and Class B much more *dicoccum*-like than Class C. The proportion of *vulgare*-like character was 16, 20 and 32% for Classes A, B and C, respectively. Considering the character of the individual plants, the ratios of *dicoccum*-like to *vulgare*-like were 12.1:1, 5.3:1, and 2.2:1 for classes A, B and C, respectively. Furthermore, the *vulgare*-like plants of classes A and B were less *vulgare*-like than those of Class C. There were present, however, in Classes A and B some plants that were fully as *vulgare*-like as any in Class C. These results indicate that, in an interspecific wheat cross, the breeder should give special attention to the shrunken F_2 seeds if he has very limited nursery space and a large amount of seed; whereas if he has plenty of space for a large population, special care of shrunken F_2 seeds does not seem warranted.

It has been commonly observed that wide interspecific crosses in wheat give rise to F_2 seed that varies greatly in endosperm development. These seeds are all produced by F_1 plants that are about as uniform as the plants of either parent variety, providing the latter were reasonably pure when the cross was made. The F_2 seeds vary in endosperm development from badly shrunken individuals carrying little or no food supply to individuals that are fully as plump as the seed of either parent variety. When this seed is sown in a nursery under ordinary field conditions, most of the very shrunken individuals fail to produce plants. Since cytological evidence indicates that an unbalanced chromosome condition may cause a shrunken endosperm which is known to affect plant characters, it is important to determine the relationship between endosperm development and plant type in wide wheat crosses. Such a determination should show the significance of the field elimination of individuals with shrunken endosperms.

The present investigation was made for the purpose of studying the relationship between the degree of plumpness (as indicating endosperm development) of F_2 seeds and the type of the resulting F_2 plants in interspecific wheat crosses.

[1] *Manuscript received May 26, 1931.*
Contribution from the laboratories of the University of Saskatchewan, Canada, with financial assistance from the National Research Council of Canada. This study forms a part of a co-operative attack on the problem of cereal rust in Canada, carried on jointly by the National Research Council, the Federal Department of Agriculture and the Universities of Manitoba, Saskatchewan and Alberta. The results were reported in full at the meeting of the Associate Committee on Field Crop Diseases at Winnipeg on April 9, 1931.

[2] *Professor of Field Husbandry, University of Saskatchewan.*

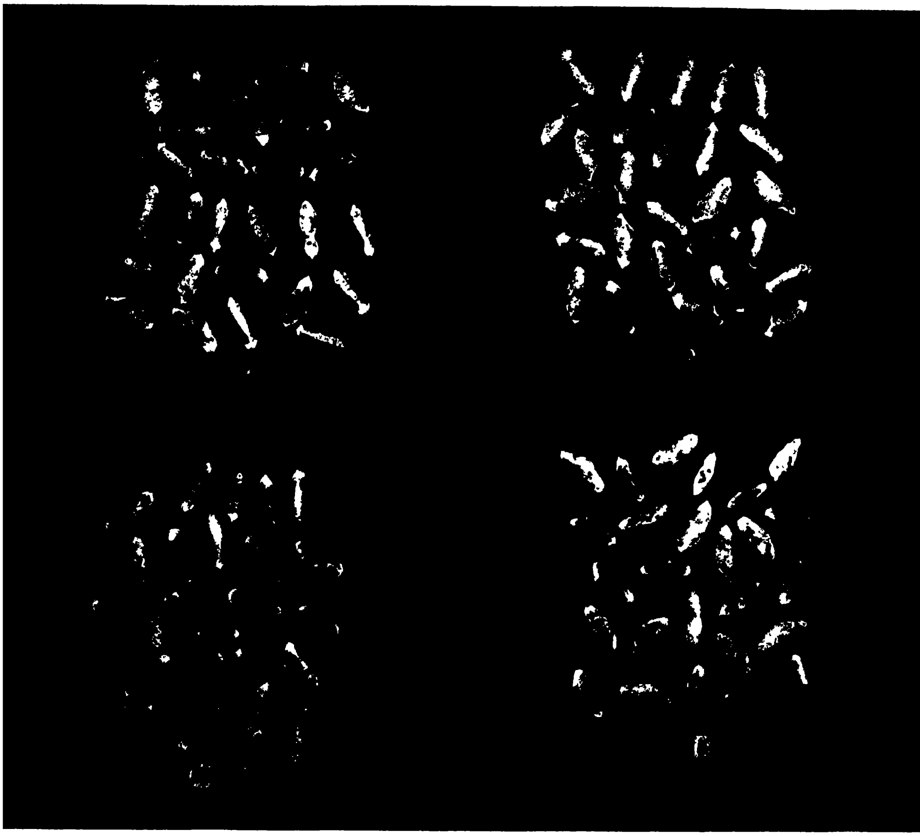


FIG. 1. *Vernal × Marquis F_2 seed. A—random sample; B—plump (Class A); C—slightly shrunken (Class B); D—shrunken (Class C).*

It was decided to limit the study to an emmer × common cross, since such crosses have been worked with extensively both genetically and cytologically. The cross Vernal (*T. dicoccum*) × Marquis (*T. vulgare*) appeared to be highly desirable for this study, for it had been studied recently by Thompson and Hollingshead (4), Harrington (1) and by Harrington and Smith (2). These studies furnished a background of cytological and genetical information. In addition, this cross was being used in the writer's project for the attainment of desirable rust resistant wheat, and much nursery, greenhouse and laboratory data were available.

Procedure

A random sample of several hundred F_2 seeds from the cross Vernal (Sask. 1289)* × Marquis (Sask. 1221)* was divided into three classes based upon kernel plumpness as follows: (1) plump, (2) slightly shrunken, (3) shrunken. The sample consisted of 55.5% plump seeds, 41.0% slightly shrunken and 3.5% shrunken. Fig. 1 illustrates the random sample and its three component parts. Eighty seeds of each plumpness group were sown in the nursery in May 1929 in rows one foot apart and spaced six inches apart in the rows. Percentage emergence was recorded for the three classes of F_2 and the parent variety checks. At maturity the separate groups were harvested by pulling the plants.

A laboratory study was made of the plants with respect to characters in which the parent varieties differed markedly. These were as follows:

Spike form (width ratio of lateral side to dorsi-ventral side)
Spike compactness (average length of ten central internodes)
Stem hollowness (taken 2 cm. below the collar)
Stem thickness (taken 2 cm. below the collar)
Lower width of rachis segment
Rachis hairiness (marginal)
Glume adherence
Glume shape (length *vs.* width)
Glume shoulder width
Keel sharpness (angle shown by cross section of lower half)
Awning
Seed character (considering kernel length and width, cheek angularity and crease width and depth).

A full description of the parent variety stocks and of the F_1 was published by the writer (1) in 1930. Only the classes "slightly shrunken" and "shrunken" were studied morphologically, owing to the general similarity between the plants of the groups "plump" and "slightly shrunken".

The results from the 1929 study were so interesting that it was thought worth while to repeat the work in 1930 using F_2 seed from the same lot as was used in 1929. This was done in the same manner as in 1930, but the number of seeds sown was larger, as follows: 100 plump, 125 slightly shrunken, 155 shrunken. The increase in number for the slightly shrunken and shrunken

These parental variety stocks were known to be highly uniform.

classes was to offset the poorer stands expected from these classes. The harvested plants of all three classes were studied morphologically. For convenience the three groups of plants from plump, slightly shrunken and shrunken seed, respectively, will be referred to hereafter as Class A, Class B and Class C.

Results

Seedling Emergence

The seedling emergence for both studies is shown in Table I. The percentage germination for the classes "plump" and "slightly shrunken" are fairly similar and not much lower than that for the parent varieties. Evidently slight shrunkenness of the seed is not a serious limiting factor to the survival of F_2 seedlings. On the other hand, the stand from shrunken seeds was very poor in both tests, averaging only 35.5%.

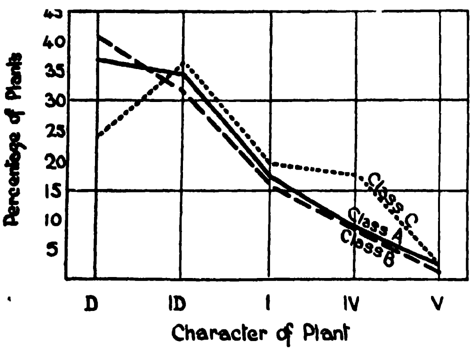


FIG. 2. *Spike form in Vernal* × *Marquis* F_2.

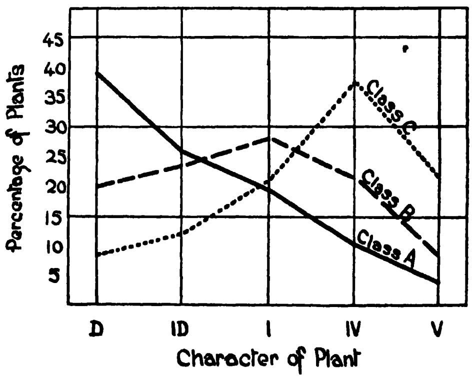


FIG. 3. *Rachis width in Vernal* × *Marquis* F_2.

TABLE I

COMPARATIVE DATA ON SEEDLING EMERGENCE

Year of study	Percentage emergence				
	Marquis	Vernal	F_2 plants from seeds that were:		
			Plump Class A	Slightly shrunken Class B	Shrunken Class C
1929	77	75	64	60	37
1930	66	60	64	56	34

Comparison of Classes A, B and C for Morphologic Characters

The results of the laboratory study of 1929 material showed that Class B was more *dicoccum*-like than Class C with respect to eleven of the twelve characters used and that, conversely, Class C was more *vulgare*-like than Class B for nine of the twelve characters.

Essentially the same results were obtained in the 1930 study as in the previous work as far as the comparison between Class B and Class C is concerned. In addition there were the Class A results. As expected from the superficial

examination in the field in 1929, Classes A and B were found not to differ much. Nevertheless the data reveal a definite tendency for Class A to be more *dicoccum*-like and Class B more *vulgare*-like for most of the characters studied. Comparing Classes B and C, it was found that Class B was more *dicoccum*-like than Class C for every character, and that Class C was more *vulgare*-like than Class B for ten of the twelve characters. Descriptions of random groups of plants from each plumpness class of the 1931 material are given in Table II.

TABLE II

DESCRIPTION OF A RANDOM PORTION OF THE VERNAL × MARQUIS F_2 PLANTS FROM EACH OF THE THREE PLUMPNESS CLASSES STUDIED IN 1930

Plumpness class	Line No.	Spike form	Spike compactness	Stem hollowness	Rachis articulation	Rachis width	Stem thickness	Glume adherence	Rachis hairiness	Shoulder width	Glume length-width ratio	Keel sharpness	Awning	Seed character*
A	62	D**	D	D	ID	ID	I	ID	D	D	D	D	D	
A	63	ID	IV	I	D	ID	I	ID	D	D	D	D	I	
A	64	V	V	I	V	IV	ID	V	V	D	ID	IV	D	
A	65	D	D	D	ID	D	D	IV	ID	D	D	ID	D	
A	66	D	D	ID	D	ID	I	ID	D	I	I	D	IV	
A	67	ID	D	D	ID	IV	V	I	D	I	I	I	V	
A	68	I	D	IV	ID	I	IV	IV	D	D	D	V	ID	
A	69	ID	D	D	ID	D	ID	D	ID	ID	D	D	V	
A	70	I	V	I	D	I	IV	I	IV	I	ID	V	V	
A	71	I	D	ID	D	I	ID	I	D	ID	D	IV	D	
A	72	D	D	D	ID	ID	I	I	D	I	I	I	V	
A	73	ID	D	D	ID	ID	ID	ID	D	D	D	D	V	
A	74	D	D	ID	ID	D	ID	V	D	ID	I	I	ID	
A	75	D	D	D	ID	IV	IV	IV	ID	D	ID	I	V	
A	76	ID	D	ID	D	D	D	D	D	ID	D	D	V	
A	77	I	D	I	ID	I	I	I	ID	D	I	I	V	
A	78	ID	D	IV	D	V	IV	D	I	IV	IV	ID	IV	
A	79	ID	D	ID	ID	D	D	I	D	ID	I	IV	IV	
A	80	I	D	ID	ID	ID	I	I	ID	ID	IV	ID	ID	
A	81	IV	D	ID	I	ID	I	I	D	D	I	ID	D	
B	108	D	D	IV	D	IV	IV	D	IV	IV	I	ID	IV	IV
B	109	ID	D	ID	ID	ID	ID	IV	D	D	D	D	I	ID
B	110	I	D	ID	ID	D	ID	IV	I	D	ID	I	ID	IV
B	111	D	D	IV	IV	I	I	IV	I	ID	I	D	D	I
B	112	I	D	I	IV	I	ID	I	ID	ID	I	ID	I	IV
B	113	D	D	ID	D	D	D	I	D	D	D	D	D	ID
B	114	IV	V	IV	ID	IV	V	I	ID	D	D	I	D	IV
B	115	V	V	IV	V	V	IV	V	IV	D	ID	IV	D	IV
B	116	ID	D	ID	D	I	I	D	D	ID	ID	D	D	ID
B	117	ID	D	D	ID	IV	I	I	D	D	ID	D	IV	ID

**Seed character was not recorded for Class A plants.*

***D = dicoccum-like; V = vulgare-like; I = intermediate; ID = intermediate between D and I; IV = intermediate between I and IV.*

TABLE II—*Continued*

Plumpness class	Line No.	Spike form	Spike compactness	Stem hollowness	Rachis articulation	Rachis width	Stem thickness	Glume adherence	Rachis hairiness	Shoulder width	Glume length - width ratio	Keel sharpness	Awning	Seed character
B	118	I	D	IV	I	I	IV	IV	V	D	D	V	D	ID
B	119	D	D	ID	I	ID	D	I	D	D	D	I	D	ID
B	120	IV	D	IV	ID	I	I	V	I	D	ID	V	ID	ID
B	121	D	D	ID	IV	D	D	ID	D	D	D	ID	V	D
B	122	ID	I	D	D	ID	D	I	ID	ID	ID	I	D	ID
B	123	I	D	ID	I	IV	V	IV	D	D	D	V	I	ID
B	124	D	D	ID	IV	I	I	ID	D	ID	ID	D	IV	I
B	125	D	IV	I	D	ID	D	D	D	ID	D	D	D	D
B	126	ID	D	ID	I	D	ID	I	ID	ID	I	ID	IV	I
B	127	ID	D	I	D	D	I	ID	D	I	I	I	V	IV
C	158	ID	ID	I	D	V	IV	ID	V	IV	IV	ID	D	I
C	159	ID	ID	IV	ID	D	I	I	I	I	I	IV	IV	D
C	160	I	D	V	V	IV	IV	V	ID	I	I	I	V	IV
C	161	I	D	V	IV	V	V	IV	ID	IV	V	IV	D	V
C	162	ID	D	D	ID	ID	D	ID	D	D	D	ID	V	ID
C	163	D	D	D	ID	ID	I	IV	D	D	D	D	IV	ID
C	164	I	I	I	ID	IV	IV	I	IV	ID	ID	ID	D	I
C	165	D	D	ID	D	D	D	ID	ID	D	D	D	D	D
C	166	IV	IV	I	I	I	V	IV	ID	ID	I	IV	IV	IV
C	167	I	D	IV	I	IV	V	I	ID	IV	IV	I	IV	I
C	168	ID	D	I	IV	I	D	V	D	D	ID	I	IV	I
C	169	D	D	I	IV	I	I	I	D	IV	IV	I	IV	I
C	170	ID	D	I	D	ID	ID	ID	D	I	I	D	V	ID
C	171	V	IV	IV	IV	I	IV	V	ID	ID	I	V	IV	IV
C	172	ID	ID	I	I	IV	ID	IV	ID	ID	ID	I	IV	ID
C	173	IV	D	IV	ID	V	V	IV	V	V	V	I	IV	I
C	174	D	D	IV	ID	V	V	I	D	I	V	I	V	IV
C	175	ID	I	IV	ID	I	ID	IV	I	ID	D	ID	I	†
C	176	IV	D	I	I	I	I	ID	I	ID	ID	I	D	ID
C	177	I	D	ID	ID	D	D	V	D	D	D	IV	ID	D

†*Sterile*

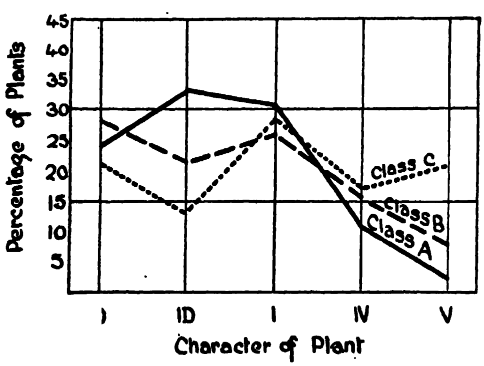


FIG. 4. *Stem thickness in Vernal × Marquis F_3.*

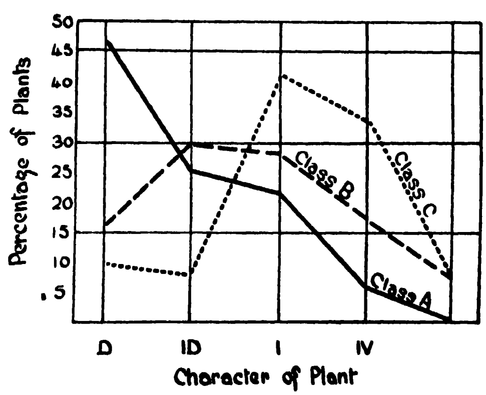


FIG. 5. *Stem hollowness in Vernal × Marquis F_3.*

TABLE III

DISTRIBUTION OF THE 46 VERNAL x MARQUIS F_2 PLANTS FROM *plump* SEEDS (CLASS A) WITH RESPECT TO EACH MORPHOLOGIC CHARACTER

Character	Character categories				
	D	ID	I	IV	V
Spike form	17	16	8	4	1
Spike compactness	40	0	1	1	4
Stem hollowness	21	12	10	3	0
Rachis articulation	14	23	6	1	2
Rachis width	18	12	9	5	2
Stem thickness	11	15	14	5	1
Glume adherence	6	8	12	15	5
Rachis hairiness	30	11	3	1	1
Shoulder width	27	13	5	1	0
Glume shape	23	12	9	2	0
Keel sharpness	16	8	9	5	8
Awning	15	7	2	6	16

TABLE IV

DISTRIBUTION OF THE 90 VERNAL x MARQUIS F_2 PLANTS FROM *slightly shrunken* SEEDS (CLASS B) WITH RESPECT TO EACH MORPHOLOGIC CHARACTER

Character	Character categories				
	D	ID	I	IV	V
Spike form	37	29	15	8	1
Spike compactness	58	8	10	5	9
Stem hollowness	14	27	26	16	7
Rachis articulation	28	19	23	15	5
Rachis width	18	21	25	19	7
Stem thickness	26	20	23	14	7
Glume adherence	22	18	25	15	10
Rachis hairiness	40	22	11	8	9
Shoulder width	51	22	13	3	1
Glume shape	37	34	15	3	1
Keel sharpness	32	21	18	10	9
Awning	25	8	17	28	12
Seed character	17	31	22	19	1

Since the results of the 1929 work and of the duplicate study made in 1930 agree reasonably well, they may be combined for the purpose of summarization. This has been done in Tables III, IV, and V. The results on four important species differentiating characters are presented graphically in Fig. 2 to 5. In Fig. 6 the proportion of *vulgare*-like character that occurred in each plumpness class is shown for both 1929 and 1930. This graph illustrates quite clearly (1) the close agreement between the results of the two studies and (2) the distinct relationship between endosperm development and plant character. The 1930 study wherein all three classes were studied shows 16, 20 and 32% of *vulgare*-like* character for the Classes A, B and C, respectively.

**Categories IV and V were taken collectively as showing vulgare-like character.*

TABLE V

DISTRIBUTION OF THE 61 VERNAL × MARQUIS F_2 PLANTS FROM *shrunken* SEEDS (CLASS C) WITH RESPECT TO EACH MORPHOLOGIC CHARACTER

Character	Character categories				
	D	ID	I	IV	V
Spike form	15	22	12	11	1
Spike compactness	24	10	15	7	5
Stem hollowness	6	5	25	20	5
Rachis articulation	17	18	12	10	4
Rachis width	5	7	13	23	13
Stem thickness	13	8	17	10	13
Glume adherence	8	13	14	15	11
Rachis hairiness	22	16	9	5	9
Shoulder width	17	18	13	11	2
Glume shape	11	15	20	9	6
Keel sharpness	14	16	18	6	7
Awning	14	6	5	23	13
Seed character	5	17	17	7	2

Character of Individual Plants with Respect to Classes A, B and C

The foregoing analysis of the results has been upon the basis of population characteristics and tendencies, but it is also important to consider the data from the viewpoint of individual plant character. This was done by taking ID and D as indicating *dicoccum*-like character and IV and V as representing *vulgare*-like character. Category I, being a neutral class, was not considered. A determination was made of the relative proportions of *dicoccum*-like and *vulgare*-like plants in each plumpness class for the 1929 and 1930 studies. The combined results are shown in Table VI. The average ratio of *dicoccum*-like to *vulgare*-like plants was 12.1 to 1.0 for Class A, 5.3 to 1.0 for Class B and 2.2 to 1.0 for Class C.

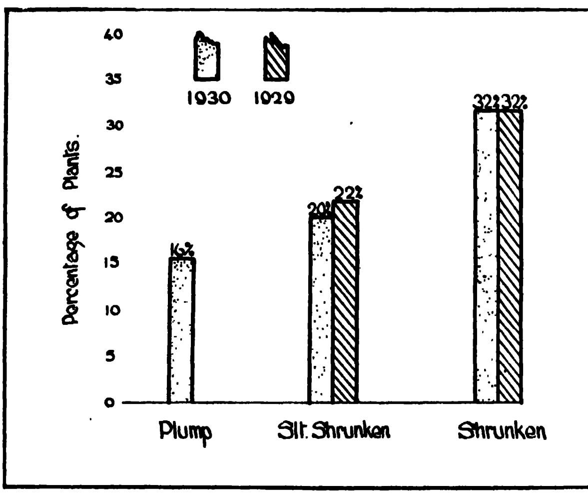


FIG. 6. *Proportion of vulgare-like character in a dicoccum × vulgare F_2 from seeds having different degrees of endosperm plumpness.*

It is apparent from these results that the ratio of *dicoccum*-like to *vulgare*-like plants depends upon the plumpness of the F_2 seed,—the plumper the seed the more *dicoccum*-like the resulting plants.

Since the *vulgare*-like plants are not equally *vulgare*-like it is of interest to examine the character of these plants to determine the relationship between

the degree of *vulgare*-ness and the plumpness of the F_2 seeds. In Table VII the data on the *vulgare*-like plants are summarized according to the three plumpness classes, and in each case the ratio of D plus ID to IV plus V determined. These ratios are 1.0 to 1.9 for Class A, 1.0 to 2.0 for Class B and 1.0 to 2.8 for Class C. It is evident that the *vulgare*-like plants of Class A were less *vulgare*-like than those of Class B, and much less *vulgare*-like than those of Class C.

The questions then arise: Are the *vulgare*-like plants of Classes A and B *all* less *vulgare*-like than those of Class C, or, if not, are they consistently unlike *vulgare* in certain characters and in that way different from the Class *C vulgare*-like plants? Examination of the data from all of the *vulgare*-like plants showed that both of these questions could be answered in the negative. Some of the most *vulgare*-like plants in the study were in Class B and the *dicoccum* resemblances that existed in the *vulgare*-like plants appeared to be distributed at random.

TABLE VI

THE DETERMINATION OF THE PROPORTION OF *vulgare*-LIKE F_2 PLANTS FROM F_2 SEEDS HAVING DIFFERENT DEGREES OF ENDOSPERM PLUMPNESS

RESULTS FROM THE RANDOM F_2 GROWN IN 1929 AND 1930

Basis of classification of F_2 plants	Distribution of F_2 plants according to F_2 seed plumpness					
	Class A (plump)		Class B (sl. shrunk)		Class C (shrunken)	
	No. of plants	Ratio of *dicoccum*-like to *vulgare*-like plants	No. of plants	Ratio of *dicoccum*-like to *vulgare*-like plants	No. of plants	Ratio of *dicoccum*-like to *vulgare*-like plants
Plants classified as D, ID or I for all of the 12 characters	9		8		2	
Plants classified as V, IV or I for all of the 12 characters	0		0		1	
Plants classified as D or ID for at least two more characters than as IV or V	41	13.6 : 1.0	67	5.6 : 1.0	33	2.2 : 1.0
Plants classified as V or IV for at least two more characters than as ID or D	3		12		15	
Plants classified as D or ID for more characters than as IV or V	42	10.5:1.0	73	4.9 : 1.0	40	2.2 : 1.0
Plants classified as V or IV for more characters than as ID or D	4		15		18	
Average ratio for last two sets of determinations*		12.1 : 1.0		5.3 : 1.0		2.2 : 1.0

**The numbers in the first set of determinations are too small to be accurate.*

TABLE VII

THE RELATIONSHIP BETWEEN F_2 SEED PLUMPNESS AND THE DEGREE OF *vulgare*-LIKE CHARACTER IN THE *vulgare*-LIKE F_2 PLANTS

Plumpness class	No. of *vulgare*-like F_2 plants	Character categories					Ratio of *dicoccum*-like to *vulgare*-like character
		D	ID	I	IV	V	
A	4*	10	5	7	12	14	1.0 : 1.9
B	15	23	25	46	59	38	1.0 : 2.0
C	18	25	20	61	70	56	1.0 : 2.8

***This number is small owing to no Class A plants being studied in 1929.*

Discussion and Conclusions

The fact that the shrunken F_2 seeds of the cross *T. dicoccum* × *T. vulgare* tend to give plants that are more *vulgare*-like than those from plump or slightly shrunken seeds is of considerable interest to the plant breeder. If he has very limited field space in which to grow an F_2 population from an interspecific cross and has a large amount of seed available, it would appear advisable not to select the plumpest portion of the seeds but rather the shrunken portion. The low viability of the shrunken seeds might be compensated for by sowing at a heavier rate. At the same time, all possible care to insure emergence and further growth of the young seedlings should be taken. Seedlings from shrunken seeds might even be started in a greenhouse.

On the other hand, if the breeder is not especially limited as to nursery space and has only a reasonably large supply of seed, which is usually the case, the findings of this study indicate that he need give no special attention to the shrunken seeds. This is apparent from the calculation made in Table VIII. These calculations were made for the purpose of obtaining figures that would represent the values of the three plumpness classes in terms of comparable *vulgare*-like plants. The final value for each class was arrived at by multiplying together the figures in the 2nd, 3rd, 4th and 5th columns and bringing the resulting figures to a percentage basis. The method used is logical and takes fully into account the different quantitative and qualitative attributes of the

TABLE VIII

A VALUATION OF THE IMPORTANCE OF SHRUNKEN F_2 SEEDS IN THE CROSS VERNAL EMMER × MARQUIS

Plumpness class	Proportion in random sample, %	Emergence in field, %	*Vulgare*-like plants		Percentage values
			Proportion, %	Ratio V & IV : D & ID	
A	55.5	64.0	7.6	1.9 : 1.0	37.2
B	41.0	58.0	15.9	2.0 : 1.0	54.9
C	3.5	35.5	31.3	2.8 : 1.0	7.9

three classes. The shrunken seeds gave proportionately a much higher number of *vulgare*-like plants than either the plump or the slightly shrunken seeds, and these *vulgare*-like plants averaged nearly 50% higher in number of *vulgare* characters than those from the other classes. But these advantages are much more than balanced by the very low proportion of shrunken seeds to start with and the low emergence in the field. Consequently under ordinary field conditions the expectation of comparable *vulgare*-like plants from the shrunken seeds would be only one-twelfth of that from the plump and slightly shrunken seeds. That is to say, the results demonstrate that the shrunken seeds in the cross studied were of relatively little importance.

If the breeder has only a small amount of F_2 seed the situation is different, because the plants from shrunken seeds are in general more heterozygous than those from plump seeds. A given lot of plants from shrunken seeds should yield progeny with a greater proportion of diversity in character combinations than a similar number of plants from plump seeds. Owing to this fact every feasible means should be used to get the shrunken seeds to produce plants *in cases where the total amount of F_2 seed is very limited.* In such cases rigid selection of plants would not be made until the F_3 generation.

The results of the present study are of particular interest in connection with the method used by McFadden (3) on the cross Yaroslav (*T. dicoccum*) × Marquis (*T. vulgare*), a cross not unlike the one used in the present study. McFadden stated that he used a Clipper seed cleaner vigorously upon all the threshed seed obtained from the F_3 population* grown *en masse* in his nursery. He added that the grading process and strong air blast removed practically all of the light weight seed. He then used for his next year's nursery only the plump, well-developed grains. It is apparent that he discarded a very large proportion of the shrunken seeds, yet he was remarkably successful and produced the well-known highly rust resistant bread wheat varieties, Hope and H-44.

Genetically and cytologically the appearance of a much larger proportion of *vulgare*-like plants from the shrunken seed than from the plump or slightly shrunken seeds is to be expected. Thompson and Hollingshead (4) showed clearly for a *dicoccum* × *vulgare* cross, similar to the one used by the writer, that after the F_1 generation surviving individuals with unbalanced chromosome conditions were more or less abnormal in various characters. Of these the ones that approached either a 28- or a 42-chromosome condition were the least abnormal. Now the number of hybrids approaching the 28-chromosome condition was much larger than the number that approached *vulgare* in chromosome constitution. This is to be expected, since chromosome loss or elimination is found to occur frequently in such a cross and the restitution of hybrids with 42 or nearly 42 chromosomes is much less likely to occur than the restitution of hybrids with 28 or nearly 28 chromosomes. Therefore, since the plumper seeds have the closest approach to a balanced chromosome condition, they should give a larger proportion of *dicoccum*-like plants than of

**No seed or plant selection was made in F_2 owing to the very limited size of the population.*

vulgare-like plants. Conversely, the shrunken seeds should yield a relatively higher proportion of *vulgare*-like plants. Of course, *some* plump seeds should give rise to *vulgare*-like plants and *some* shrunken seeds should give *dicoccum*-like plants. The results fitted these expectations quite satisfactorily.

References

1. HARRINGTON, J. B. The relationship between morphologic characters and rust resistance in a cross between emmer (*Triticum dicoccum*) and common wheat (*T. vulgare*). Can. J. Research, 2: 295-311. 1930.
2. HARRINGTON, J. B. and SMITH, W. K. The inheritance of reaction to black stem rust of wheat in a *dicoccum* x *vulgare* cross. Can. J. Research, 1: 163-188. 1929.
3. McFADDEN, E. S. A successful transfer of emmer characters to *vulgare* wheat. J. Am. Soc. Agron. 22: 1020-1034. 1930.
4. THOMPSON, W. P. and HOLLINGSHEAD, L. Preponderance of *dicoccum*-like characters and chromosome numbers in hybrids between *Triticum dicoccum* and *Triticum vulgare*. J. Genetics, 17: 283-307. 1927.

WALLROTHIELLA ARCEUTHOBII, A PARASITE OF THE JACK-PINE MISTLETOE[1]

By E. Silver Dowding[2]

Abstract

Arceuthobium, the host of *Wallrothiella Arceuthobii*, has been found in British Columbia, Alberta, Manitoba, and Ontario and *Wallrothiella Arceuthobii* has been found in Manitoba and Alberta.

Arceuthobium fruits become infected in Canada in the spring, about a week after fertilization. The fungus and the infected fruits then increase in size, and they attain their maximum development by the summer of the following year.

The ascospores are not violently discharged into the air. The spores ooze out into water when the perithecia are wet.

The mature perithecium is made up of two compartments, the lower compartment containing the asci, and the upper compartment into which the ascospores are discharged and where they collect.

It is suggested that insects are agents which disperse the ascospores.

Ascospores sown in *Arceuthobium* decoction commence to germinate, but growth ceases after the germ tube has reached the length of about one millimetre.

Attempts to inoculate the stigmas of healthy Arceuthobium with "sprout-mycelium" have so far been unsuccessful.

I. Introduction

Arceuthobium is a mistletoe, allied to Viscum and Loranthus, which parasitizes certain conifers. Several species of the genus, in their turn, are parasitized by a fungus known as *Wallrothiella arceuthobii* (Pk.) Sacc. Arceuthobium is dioecious and the male and female plants are usually found growing on the same tree. The fungus attacks the pistillate flowers only and therefore occurs exclusively on the female plants.

The writer has paid special attention to *W. arceuthobii* as it grows on *Arceuthobium americanum* on *Pinus banksiana* in western Canada.

Arceuthobium americanum, when occurring on *P. banksiana*, is commonly known as the jack-pine mistletoe. This mistletoe induces abnormal branching of the infected jack pines resulting in the formation of "witches' brooms." In the field it is not difficult to find these brooms, for they stand out clearly against the sky (Plate I, Fig. 1 and 2).

To find *W. arceuthobii* one must first find "witches' brooms" like those just described. When these have been discovered, one must examine the fruits of the female mistletoes. If the fruits have been parasitized by the fungus, the fungus can readily be recognized as a black stroma protruding from their apices. The protruding part of each stroma is more or less hemispherical, is about 1 mm. in length and 1.5 mm. in width, and exhibits about forty papillae which are the protruding necks of as many perithecia (Text-fig. 1, 3, 4).

The jack-pine mistletoe severely damages the pines which it attacks, in that it checks their growth and renders their timber valueless for commercial purposes. At Victoria Beach, Lake Winnipeg, in certain stands of *Pinus*

[1] *Manuscript received May 15, 1931.*
Contribution from the Department of Botany, University of Manitoba, Winnipeg, Canada.

[2] *Research worker on Dermatophytes, University of Manitoba, and holder, at the time, of the Hudson's Bay Research Fellowship.*

banksiana, it was observed that at least four-fifths of the fruits of the mistletoe were parasitized by *Wallrothiella*. Since parasitized fruits never produce viable seeds, it is clear that the fungus is one of the agencies which serves to restrict the spread of the mistletoe in North America.

Wallrothiella arceuthobii was discovered in 1873, in New York State by Peck (11) who named it *Sphaeria arceuthobii*. In 1900 it was observed in the Upper Peninsula of Michigan by Wheeler (13), in 1915 in Montana and Idaho by Weir (12), and in 1929 in Alberta, Canada, by the author. These records of collections from such widely separated localities seem to indicate that *W. arceuthobii* is widely distributed throughout the northern part of North America and that possibly it occurs there from the eastern to the western coast.

Weir (12) reports that *Wallrothiella arceuthobii* has been observed in the United States of America on the following five species of *Arceuthobium:*

A. pusillum (Peck) Kuntze on *Picea mariana*,
A. americanum (Nutt.) Kuntze on *Pinus murrayana*,
A. douglasii (Engelm.) Kuntze on *Pseudotsuga taxifolia*,
A. douglasii var. *abietina* Engelm. on *Abies grandis* and *A. lasiocarpa*.
A. douglassii var. *microcarpa* Engelm. on *Picea engelmanni*.

In this paper an attempt will be made to extend our knowledge of the distribution, life history, and ecology of *Wallrothiella arceuthobii* as it occurs on the jack-pine mistletoe in Canada.

II. Distribution in Canada

Dr. E. H. Moss first drew the writer's attention to *Wallrothiella* on *Arceuthobium* fruits collected from pines in central Alberta. During the last three years the author has taken every opportunity to examine the various species

TABLE I

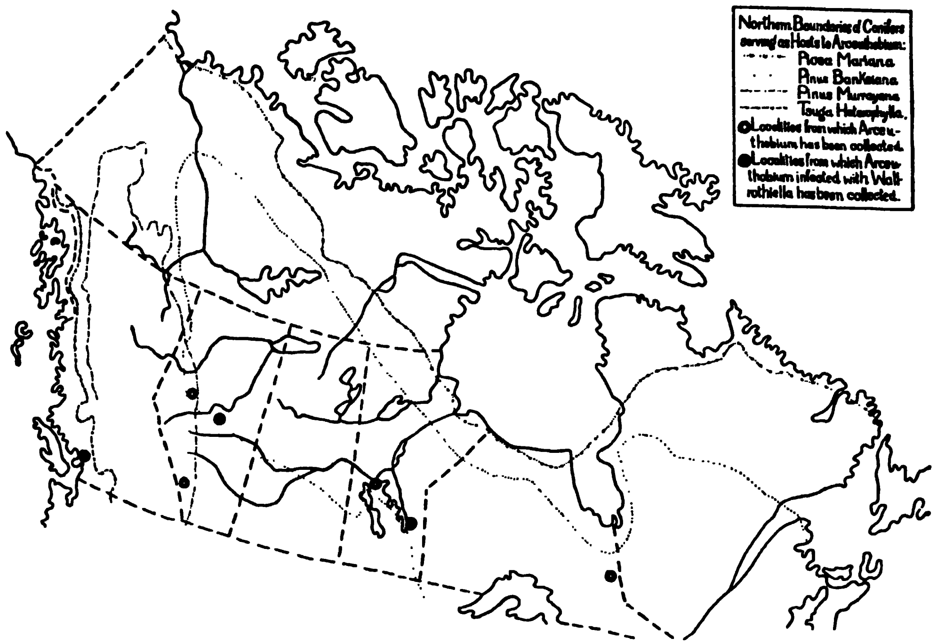
AREAS IN CANADA FROM WHICH ARCEUTHOBIUM HAS BEEN COLLECTED

Locality	Mistletoe	Host tree	Infection by *W. Arceuthobii*
1. Mt. Garibaldi, near Vancouver	*A. tsugensis*	*Tsuga heterophylla*	Uninfected
2. Tunnel Mt., Banff, Alberta	*A. americanum*	*Pinus murrayana*	Uninfected
3. Assiniboine River, from Whitecourt to Ft. Assiniboine	*A. americanum*	*P. banksiana*	Uninfected
4. North of Edmonton between N. Saskatchewan River and Athabasca River	*A. americanum*	*P. banksiana*	Infected
5. Grand Rapids at the mouth of the Saskatchewan River	*A. americanum*	*P. banksiana*	Uninfected
6. Victoria Beach on the S.E. shore of Lake Winnipeg	*A. americanum*	*P. banksiana*	Infected
7. Near Lake Temagami, eastern Ontario	*A. pusillum*	*P. mariana*	Uninfected

The trees illustrated are Pinus banksiana. The mistletoe is Arceuthobium americana. FIG. 1. *Pine in central Alberta, infected with Arceuthobium. The infected limb shows enormous hypertrophy.* FIG. 2. *Pines in central Manitoba infected with Arceuthobium. The trees are dwarfed and abnormally compact in habit.* FIG. 3. *A pine branch infected with female plants of Arceuthobium whose fruits are in turn infected with Wallrothiella. (Natural size.).* FIG. 4. *Female branch of Arceuthobium bearing flowers and fruits. The fruits are parasitized by Wallrothiella. Three times natural size.* FIG. 5. ***Healthy Arceuthobium flowers growing on a "witches' broom" of a pine have been inoculated with the mycelium of Wallrothiella and tied into bags.***

of *Arceuthobium* in Canada for possible infection in order to determine the northern range of *Wallrothiella arceuthobii.* Areas in British Columbia, Alberta, Manitoba, and Ontario, where coniferous trees were severely infected with the jack-pine mistletoe, were visited and the mistletoe was examined. Fruits were discovered infected with *Wallrothiella* in Alberta and in Manitoba.

Seven areas in Canada from which *Arceuthobium* has been collected are listed in Table I, together with the name of the host trees and the presence or absence of *Wallrothiella arceuthobii.*



Map showing the distribution of Arceuthobium and of Wallrothiella in Canada.

The accompanying map of Canada shows the limits of distribution of conifers that serve as hosts of *Arceuthobium* as recorded in the Atlas of Canada (1) and it shows the localities from which healthy *Arceuthobium* and *Arceuthobium* infected with *Wallrothiella arceuthobii* were collected.

Although *Wallrothiella arceuthobii* has been collected from only two districts in Canada, central Alberta and central Manitoba, these districts are so widely separated (they are about eight hundred miles apart) that it seems probable that the range of the fungus in Canada is from the eastern to the western coast.

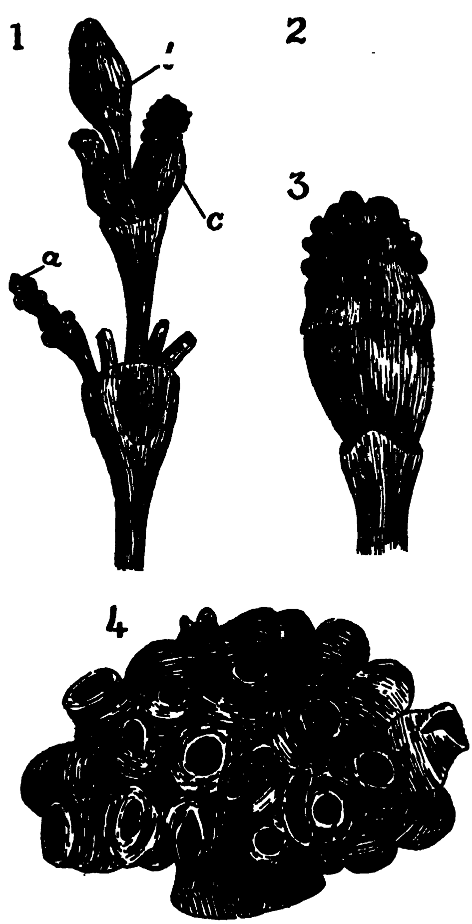
In central Alberta, where in 1927 field observations were made (3), the pines that were infected with mistletoe were growing on sandy ridges separated either by sloughs or by muskegs. The mistletoe growing on trees which bordered the depressions were the ones that were most frequently infected by *Wallrothiella.* In central Manitoba the infected trees that supported

Wallrothiella were growing on a low sandy peninsula of Lake Winnipeg. Further, Weir (12) states that he finds the fungus most frequently "in damp river bottoms or on the borders of swamp areas." From all these observations we may conclude that damp low-lying localities near water are most favorable for the growth of *Wallrothiella*.

III. Life History

The fungus invades the gynaeceum of *Arceuthobium via* the stigma. In western Canada this invasion takes place in the spring soon after pollination.

On May 8, a visit was made to a forest of mistletoe-infected *Pinus banksiana* in Alberta, when it was found that the male flowers of the mistletoe were open and exposing their pollen. Some female flowers were collected and afterwards sectioned by the paraffin method. The sections showed pollen tubes in the stylar canals. Hence on or before May 8 pollination must have been taking place.



TEXT-FIG. 1–4. *Wallrothiella arceuthobii on Arceuthobium americanum, collected in June.* TEXT-FIG. 1. *Infected female branch of Arceuthobium: a, flowers; b, fruit; c, fruit bearing Wallrothiella perithecia. Twice natural size.* TEXT-FIG. 2. *Female flowers of Arceuthobium: The perianth has been removed to show the stigma which is covered with spores of Wallrothiella. Five times natural size.* TEXT-FIG. 3. *Fruit of Arceuthobium infected with Wallrothiella. Five times the natural size.* TEXT-FIG. 4. *Stroma, cut from an infected fruit to show the perithecia. Magnification, 18.*

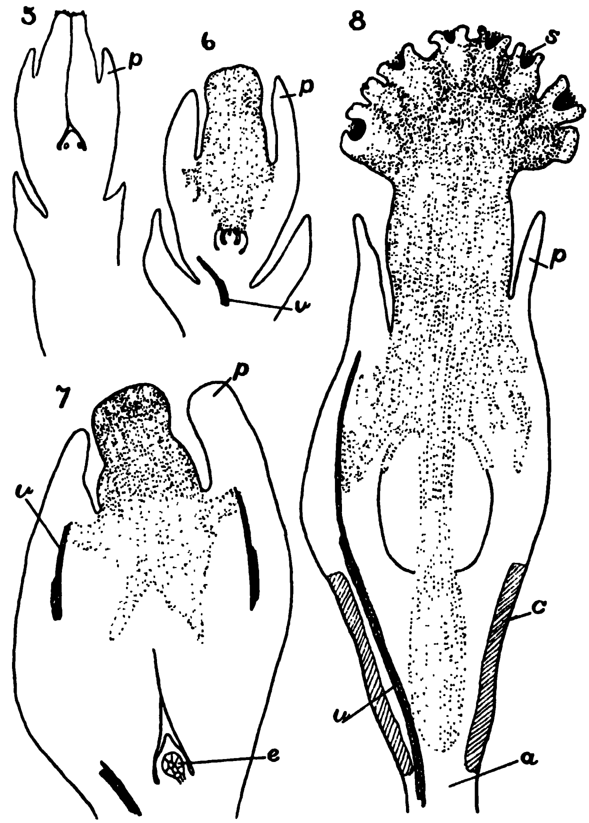
On May 24 fertilized female flowers were collected and afterwards sectioned by the paraffin method. The sections showed a small embryo contained within the nucellus and fungal tissue in the interior of the upper part of the stigma (Text-fig. 7).

The facts recorded above seem to indicate that a pollen-tube succeeds in fertilizing the ovum before the fungus has destroyed the stigma.

The fact that the pistillate flowers of *Arceuthobium* become pollinated and fertilized before they are attacked by *Wallrothiella arceuthobii* is greatly to the advantage of the fungus. Before fertilization, the flowers of *Arceuthobium* are extremely small—less than 1 mm. long—and, if they were to become infected by *Wallrothiella* at this time, the stroma of the fungus would not be able to attain its normal length of 2 mm. and its width of 1.5 mm. As soon as fertilization has been accomplished, the ovary is stimulated to grow and soon becomes 3 mm. long and many times its original size. After thus having enlarged and having become

a focus for food supply, it is very well fitted to meet all the demands made upon it by the fungus. After fertilization the infected fruit increases in size not only during the ensuing summer, but also during the summer of the next year. Throughout both summers the fungus continues its growth in the fruit, and thus the growth of the fruit and that of the fungus keep pace with one another.

After the fungus has entered the stigma, the hyphae grow down the style and enter the ovary wall (Text-fig. 6). Here they spread laterally to the vascular tissue of the perianth (Text-fig. 7 and 8). Other hyphae then grow down the central axis of the fruit in the form of a slender column (Text-fig. 8). During the spring of the second year this column replaces all the central tissue of the fruit including the embryo and grows downwards until it reaches the abscission layer of the peduncle (Text-fig. 8). In the previous autumn and during the following spring the aerial portion of the stroma comes to project as a black knob at the top of the fruit (Text-fig. 8). In this knob some forty perithecia are developed (Text-fig. 1, 3, and 4). In June, the mouths of the perithecia become distended and reveal large numbers of free ascospores (Text-fig. 4). The ascospores are dispersed from the mouths of the perithecia from early in June until late in August.



TEXT-FIG. 5–8. *Longitudinal sections of flowers and fruits of Arceuthobium americanum infected by Wallrothiella arceuthobii: p, perianth; v, vascular tissue; e, embryo; s, spores; c, collenchyma; a, abscission layer. Magnification, 14.* TEXT-FIG. 5. *Flowers bearing spores on stigma collected May 8, 1928.* TEXT-FIG. 6. *Flower with stigma replaced by fungal tissue collected June 3, 1928.* TEXT-FIG. 7. *Fruit with stigma replaced by fungal tissue. Collected July, 1928.* TEXT-FIG. 8. *Fruit completely infected with Wallrothiella, collected June 3, 1929.*

The female flower of *Arceuthobium* was described in 1888 by Johnson (8), in 1915 by Heinricher (6), and in 1931 by the author (4). It consists of a bipartite perianth which is fused to the ovary for two-thirds of its length (Text-fig. 5-8) and a gynaeceum which develops one seed in its ovary and which terminates in a hollow knob-shaped stigma. Heinricher (7) has observed that

in Austria the female flowers of *A. oxycedri* secrete an oily drop into the hollow stigma. The hollow stigma of some of the female flowers of *A. americanum* collected by the author in Manitoba in May had a glistening drop exuding from it, and doubtless this drop corresponds to that observed by Heinricher. The stigmatic secretion of *Arceuthobium* flowers may well be a suitable medium for the germination not only of pollen grains derived from the male flowers but also of ascospores of *Wallrothiella*.

The normal fruits of *Arceuthobium* violently expel their seeds to a distance of many yards (3); but abnormal fruits infected with *Wallrothiella* never discharge seeds but fall to the ground a little later than the time (September) when the normal fruits explode.

IV. The Discharge and Dispersal of the Ascospores

Experiments set up with a view to finding out whether or not the ascospores are violently shot into the air were made upon material collected in the summer and examined: (1) after being kept dry until the following January; (2) in the laboratory within a few hours of its being collected; and (3) in the field immediately after being collected. Two types of experiments were set up: (1) with a Van-Tieghem cell and (2) with a cover-glass only.

(1) A tiny branch (8 mm. long) bearing infected fruits of *Arceuthobium* was placed in a Van-Tieghem cell (Text-fig. 9) so that the stromata at the top of the fruits looked upwards toward the cover-glass. To hold the branch in the required position a copper-wire support was employed. The perithecia in the stromata were 1-3 mm. from the cover-glass. After the experiment was set up the cover-glass was examined microscopically at intervals up to 48 hr. No spores were deposited by the perithecia on the under side of the cover-glass. This result goes to show that the perithecia cannot shoot their ascospores upwards to a height of 1-3 mm.

(2) Pieces of the stroma of infected fruits were sliced off and placed on a slide so that the perithecia looked upwards, and then a cover-glass was rested on the top of the perithecia. No spores became deposited on the under side of the cover-glass.

From the results of the two series of experiments just described we may conclude that the perithecia of *Wallrothiella arceuthobii* are unable to discharge their ascospores violently.

If a mature stroma is placed in water in a beaker for four minutes, is then removed, set on a slide in air, and examined with the low power of the microscope, bubbles of air can be seen emerging from the mouths of the perithecial cavities, and bearing ascospores in the films of water which envelop them (Text-fig. 10).

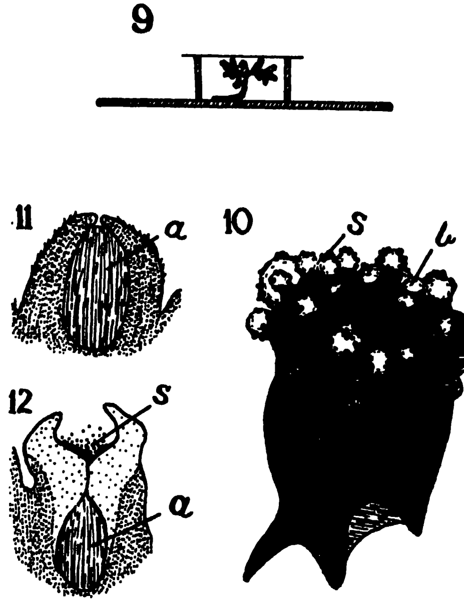
If a dry stroma collected in the summer, either immediately after having been collected or some months later, is set in water for a few minutes and is then lightly touched to white paper, it leaves a black spore-deposit on the surface of the paper.

The two observations just recorded go to show that the ascospores of the fungus are readily set free from a stroma as soon as this is wetted.

Peck (11) states that the asci of *Wallrothiella* are fugaceous, so that at first it was thought that, when the perithecia are ripe, the walls of the asci disintegrate and the ascospores thus liberated within the perithecial cavities escape from the mouths of these cavities when the stroma is wetted. That this view is not entirely correct is shown by the observations now to be recorded.

An immature perithecium, collected during the autumn or winter previous to spore dispersal, consists of a peridium four or five layers of hyphae thick which encloses a single chamber containing asci and paraphyses (Text-fig. 11). The mouth of the perithecium at this stage appears to be closed or almost so.

An examination of the perithecia in a stroma collected in the spring a year after the fungus has entered the fruit shows that the hyphae at the mouths of the perithecia have grown upwards, outwards, and then somewhat inwards so as to form a wide-open *antechamber* communicating with the original and older *perithecial cavity* by a narrow neck (Text-fig. 12).



TEXT-FIG. 9–12. *Wallrothiella arceuthobii, illustrating spore emergence: b, air bubbles; s, spores; a, asci.* TEXT-FIG. 9. *Fungus on Arceuthobium arranged in Van Tieghem cell in an attempt to obtain a spore deposit upon the cover slip. Reduced to one-half the natural size.* TEXT-FIG. 10. *Diagram of stroma soaked in water. Bubbles of air are escaping from the perithecia bringing spores out with them. Magnification, 8.* TEXT-FIG. 11. *Diagram of longitudinal section of perithecium collected in February, showing single compartment containing asci. Magnification, 35.* TEXT-FIG. 12. *Diagram of longitudinal section of perithecium collected in June, showing the lower compartment containing asci and the upper compartment containing spores. Magnification, 35.*

In a ripe perithecium, as shown in sections made by the paraffin method, the perithecial cavity always contains intact asci, and presumably also discharged asci, but never any free spores, whereas the antechamber contains free spores only (Text-fig. 4 and 12).

The observations recorded above suggest that the asci in the perithecial chamber discharge their spores into the antechamber. The means by which this is brought about is obscure. As experiments already recorded have shown, there is no reason to suppose that the asci violently shoot away their spores. It therefore seems probable that the walls of the asci become diffluent and that by their swelling they carry the spores upwards through the perithecial neck into the antechamber. An ascus has been observed greatly swollen and pressing its apex against the base of the perithecial neck. This observation seems to indicate that the asci successively discharge their eight ascospores, one ascus following another in pressing up to the base of the neck.

A stroma less than one year old, as already explained, has no antechamber. Its asci are all intact and none of them has as yet discharged its ascospores.

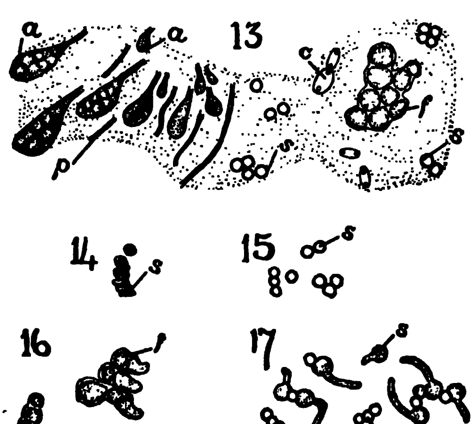
If such a stroma is placed in water for some minutes, no spores are discharged. In a mature stroma the perithecia have their antechambers filled with discharged spores. It is only from such perithecia that spores ooze out when the stroma is wetted.

The spores when dry stick together and to any substratum they may be on. Since they are not shot into the air, it is unlikely that they are dispersed by the wind. It seems not unlikely that the same insects which pollinate the flowers of *Arceuthobium* transfer the ascospores to the stigmas of the flowers. The author, in May, has seen small flies hovering over the flowers of *Arceuthobium* and ants swarming over the infected pine branches; and Mr. H. J. Brodie, whilst visiting Lake Winnipeg in August, 1930, to collect material for the writer wrote to me as follows: "I spent a considerable time in watching the movements of the ants. Each ant would crawl out to the tip of a pine needle and back to its base, repeating this operation several times on each branch tip. . . . . I have seen the ants crawling over *Arceuthobium*." Further observations are required to determine exactly whether or not ants and other insects visit the spore-filled antechambers of the perithecia and actually transport the spores from an infected fruit to a healthy flower.

Rain might splash the spores about on a single pine tree and so perhaps occasionally bring about fresh infections in the same tree. However this agency could not spread the fungus from tree to tree in a loose pine stand.

V. Asci and Ascospores

As a preliminary step in attempting to germinate the ascospores, a mature stroma that had been soaked in sterile water was rubbed on a sterile slide so as to break open the perithecia. A mucilaginous smear of fungal material was thus obtained. Microscopical examination of the smear showed that it consisted of asci, ascospores, fragments of the stroma, and sometimes foreign spores (Text-fig. 13). If a stroma is kept damp for some days, foreign spores are frequently obtained in the smear, particularly those of *Cladosporium herbarum* which are easily recognized by their amber color (Text-fig. 13).



TEXT-FIG. 13–17. *Wallrothiella arceuthobii. All the material was obtained by making a smear with a wet stroma upon a glass slide: a, asci, upside down; p, paraphyses; s, ascospores; f, fragment of stroma; c, conidia of Cladosporium. Magnification, 230.* TEXT-FIG. 13. *A typical smear from a stroma containing asci of various ages, paraphyses, fragments of the stroma Wallrothiella, ascospores, and contamination consisting of conidia of Cladosporium.* TEST-FIG. 14. *Ascospores in dry condition.* TEXT-FIG. 15. *Ascospores in water.* TEXT-FIG. 16. *Fragment of the stroma germinating in nutrient medium.* TEXT-FIG. 17. *Ascospores germinating in nutrient medium.*

Asci of various stages of development can be found in a smear from the stroma, mixed with extremely fine paraphyses. They are club-shaped, tapering below into a slender stalk, and about 25 μ long and 10 μ wide. They correspond in outline to the arrangement of the spores within. The eight spores are usually arranged in

two columns, a long column of six and a short column of two beside the longer column, so that the ascus wall is slightly extended on one side where the separated pair of spores lie (Text-fig. 13).

The ascospores are dark-colored owing to the spore wall containing a black pigment. When wet, the spores are globose (Text-fig. 15) but when dry they collapse to flattened discs. The dry spores cohere in such a way as to resemble a pile of coins (Text-fig. 14). The spores are 4 to 6 μ in diameter and, when dry, they flatten out to 2 μ in thickness.

Repeated trials were made to germinate the ascospores. The spores were sown in water, potato agar, malt agar, and pine decoction. Some were unheated, others were heated to temperatures varying from 30° to 75° C. All attempts made during the winter failed. In the spring, large quantities of fresh *Arceuthobium* plants were obtained. A decoction of *Arceuthobium* was made and the ascospores were sown in hanging drops of this medium in Van-Tieghem cells. A group of ascospores was selected and drawn with the camera lucida just after sowing. It was re-examined after 24 hr. and the identical spores that had been drawn were found to have swollen and to have commenced to germinate (Text-fig. 17). The germ tubes attained a length of about one millimetre but, unfortunately, they could not be induced to grow any further.

VI. Cultivation of Wallrothiella from the Stroma

For culturing the stroma, fresh material was used because stored material sometimes became grown over with molds. The fresh stroma was steeped for a minute in 1% corrosive sublimate, washed in sterile water, and planted in a hanging drop of sterile water or potato agar. Within a few hours, stout vigorous hyphae appeared all over the surface of the stroma (Text-fig. 18), which gave rise to large quantities of yeast-like sprout cells (Plate 2, Fig. 2 and 3).

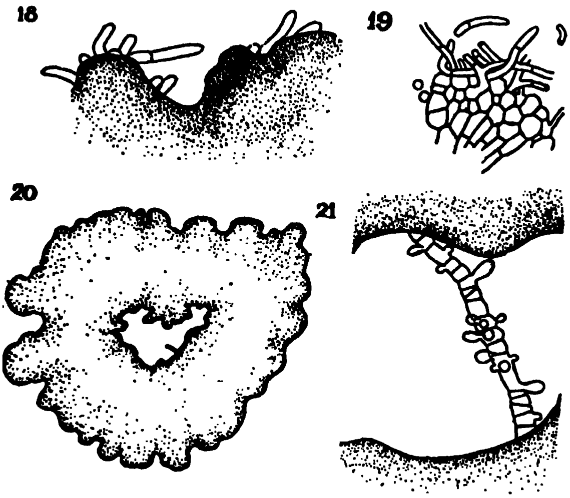
The stroma is so darkly pigmented and composed of such fine closely interwoven hyphae that even in the thinnest stroma section that it is possible to cut by hand the germinating hyphae can never be traced back to definite hyphae of the parent stroma, so that there was the possibility that the growth in the medium arose from foreign spores lodged in the crevices of the stroma.

In two sets of observations, now to be recorded, evidence was obtained which seems to show conclusively that the sprout-mycelium originated from the tissue of the stroma and not from foreign spores.

(1) A fragment of stroma was planted in a hanging drop of nutrient medium and, when a halo of hyphae had grown out into the medium, the tissue was fixed. It was then dehydrated, embedded in paraffin, sectioned 8 μ thick, and stained. The sections showed that the germinating hyphae could actually be traced back into the stroma tissue (Text-fig. 19).

(2) The stroma of *Wallrothiella* at a level where it emerges from the mistletoe fruit, is hollow. Attention was directed to the sterile inner surface of the cavity upon which no foreign spores could possibly be lodged. A large number of sections of the stroma that were cut at the level of the cavity were planted

in a hanging drop of nutrient medium, and one slice of stroma was eventually found in which the first hypha to grow took its origin from the inner surface of the cavity (Text-fig. 20; Plate 2, Fig. 1). This hypha was watched over a period of several hours, and it was seen to elongate, to become septate, and to give rise to an abundance of sprout cells (Text-fig. 21).



TEXT-FIG. 18–21. *Wallrothiella arceuthobii. Diagrams of germination of the stroma.* TEXT-FIG. 18. *Portion of the stroma sown in nutrient medium 24 hr. previously, showing outgrowth of hyphae. Magnification, 150.* TEXT-FIG. 19. *Paraffin section of stroma illustrated in Text-fig. 18., showing the connection of the germinating hyphae with the stroma tissue. Magnification, 150.* TEXT-FIG. 20. *Section of the stroma sown 24 hr. previously in nutrient medium, showing the development of a hypha from the inner surface of the cavity. Magnification, 65.* TEXT-FIG. 21. *The hypha shown in Text-fig. 20, two days later, showing formation of cross-walls and commencement of sprouting. Magnification, 150.*

The sprout cells of the *Wallrothiella* mycelium give rise by budding to more sprout cells. In a liquid medium the sprout cells bud so rapidly that a white precipitate of yeast-like cells collects at the bottom of the medium. Budding is common in the early phase of many fungi. Martin (10) has recorded that the phenomenon has been observed in the following genera: *Dermatium*, *Fumago*, *Polyspora*, *Exobasidiopsis*, *Microstroma*, *Spaceloma*, and others.

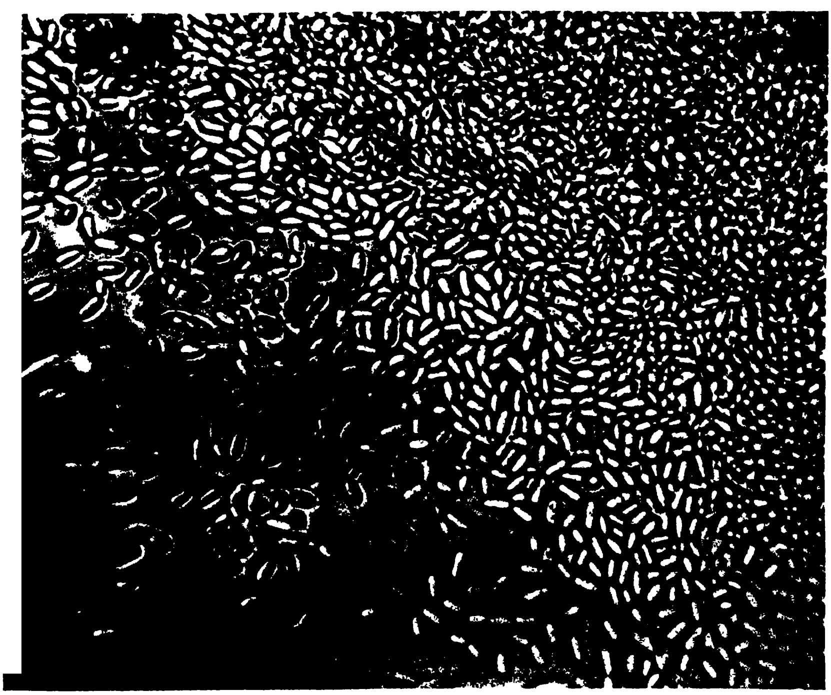
When the mycelium is older, the walls become darkly pigmented and the formation of sprout cells gives way to the formation of thick-walled conidia which clothe the hyphae in a brown sheath. The hyphae secrete sheaths of mucilage, and as the culture dries out the mucilage cements the mycelium to the substratum in which condition it can retain its vitality for at least six months. The mycelium shows a remarkable resemblance to *Dermatium pullulans*.

Brefeld (2) in 1891 describes a similar type of mycelium in a species closely related to *Wallrothiella*, *Sphaerulia intermixta*. In this species the ascospores give rise to a sprout-mycelium. After the exhaustion of the nutrient medium the sprout cells change to multicellular brown gemmae. Their ability to germinate is not injured by drying for eighteen months.

Gaümann and Dodge (5), who quote Brefeld's account, remark that "sprouting" has not been reported elsewhere in the group and that the results were probably based on impure cultures.

Strong evidence has been brought forward to show that the sprout-mycelium obtained in my cultures from stroma sections belongs to *Wallrothiella*. To establish the connection beyond any doubt it would be necessary to germinate the ascospores in pure cultures, and obtain a mycelium which would become a sprout-mycelium or to induce the sprout-mycelium obtained from a stroma to produce *Wallrothiella* perithecia.

PLATE II



All figures are those of Wallrothiella arceuthobii. FIG. 1. *Section of the stroma of Wallrothiella which was sown in potato-agar 24 hrs. previously. A hypha has grown out from the inner surface of the cavity (an arrow points to it). Magnification, 45.* FIG. 2. *Mycelium grown in potato-agar from Wallrothiella stroma. Magnification, 150.* FIG. 3. *Sprout cells in water, developed from mycelium of Wallrothiella. Magnification, 150.*

VII. Inoculation Experiments

The sprout-mycelium obtained from the stroma of *Wallrothiella* was inoculated upon healthy stigmas of *Arceuthobium*.

The first material to be used as a host in the inoculation experiment was *A. tsugensis*. This mistletoe was collected attached to *Tsuga heterophylla* and sent from Vancouver by Mrs. G. Smith to the laboratory where it was kept in water. The sprout-mycelium obtained from a stroma and growing in artificial culture was smeared on the stigmas of the mistletoe and the plants were kept for a month. The inoculations were not successful. This experiment was not regarded as conclusive because the cuticle on the stigma of *A. tsugensis* was about three times as thick as the cuticle on the stigma of *A. americanum* so that *A. tsugensis* may quite possibly be resistant to the fungus.

The following spring *A. americanum* growing on jack pine on the shore of Lake Winnipeg was used as a host. Inoculation experiments were made in the field upon plants which were left attached to the pine trees during the course of the experiment. Trees were selected for the inoculations on which there was no *Wallrothiella*. The sprout-mycelium was smeared over healthy stigmas, and the branches of inoculated fruits were covered by paper bags. In this way several hundred fruits were inoculated. Controls of fruits that had not been inoculated were also tied in bags. (Plate 1, Fig. 5).

In the following autumn the trees were re-examined and it was found that the experiments had been interfered with. Squirrels had torn most of the bags, with the exception of one, which covered about fifty fruits that had been inoculated. None of the fruits that had not been interfered with had become infected with *Wallrothiella*.

At the time of the second visit to the trees, fresh plants were inoculated with mycelium, and these are to be examined in the autumn of 1931.

Acknowledgment

This investigation was carried out in the Botanical Department of the University of Manitoba during the writer's tenure of the Hudson's Bay Research Fellowship, 1929-30. The expenses involved in collecting material were defrayed by a grant from the Royal Society. The author desires to acknowledge with her best thanks the generous assistance given her by Professor A. H. Reginald Buller.

References

1. Atlas of Canada, Dept. of the Interior, Canada. 1906.
2. Brefeld, O. Unters. Ges. Geb. Mykol. 10: 157-378. 1891.
3. Dowding, E. S. J. Ecol. 17: 82-105. 1929.
4. Dowding, E. S. Botan. Gaz. 91: 42-54. 1931.
5. Gäumann, E. A. and Dodge, C. W. Comparative morphology of fungi, McGraw-Hill. 1928.
6. Heinricher, E. Sitzb. Akad. Wiss. Wien. Abt. 1. 124 Bd. 3 & 4 heft. 181-230. 1915.
7. Heinricher, E. Sitzb. Akad. Wiss. Wien. Abt. 1. 124 Bd. 6 & 7 heft. 481-504. 1915.
8. Johnson, T. Ann. Botany, 2: 137-160. 1888.
9. MacDougal, D. T. Minnesota Botanical Studies, 2: 169-173. 1898-1902.

10. Martin, G. H. Phytophathology, 19: 1117-1123. 1929.
11. Peck, C. H. N.Y. State Museum of Natural History. 27th Ann. Rep. 1874-1876. Report of the Botanist, 73-116. 1874.
12. Weir, J. R. J. Agr. Research, 4: 369-378. 1915.
13. Wheeler, C. F. Mich. Agr. Coll. Exptl. Bull. 186: 17-28. 1900.

THE EPITHALASSA OF THE STRAIT OF GEORGIA

SALINITY, TEMPERATURE, pH AND PHYTOPLANKTON[1]

BY A. H. HUTCHINSON[2] AND C. C. LUCAS[3]

Abstract

The investigation was initiated in order to determine the extent of the Fraser River's effect on temperature, salinity, currents, and fish food, probable factors in the direction of salmon migrations. A correct valuation of the Fraser River in these respects has necessitated a survey of the Strait of Georgia waters and an estimate of the relative importance of water entering the Strait from other sources, from the sea through the passes and from other rivers. It is believed that the data presented may have a potential economic value as a basis for a further knowledge of the habits of fish, the establishment of oyster beds, the development of clam beaches and of crab and shrimp industries and the location of summer resorts. Conclusions regarding the interrelations of salinity, temperature, pH, tidal movements and phytoplankton, may be of scientific interest.

The following conclusions discussed in the paper may be emphasized: (1) The Strait of Georgia is a great basin, connected with the sea by narrow passes, which receives water from a number of large rivers, notably the Fraser River. (2) The river water is conserved during the summer and forms a marked upper layer, epithalassa, which is characterized by low salinity and high temperature. The increase in temperature as compared with sea water may amount to 10° C. or 18° F. (3) Throughout the greater part of the region this epithalassa has a stability which is sufficient to resist tidal and wave movements. (4) Since time is a factor, the heating effect of the sun, insolation, upon the epithalassa becomes most evident at regions some distance from the river mouth. In the case of a large river, as the Fraser, this distance may exceed ten miles. (5) Abundant fish food in the form of plankton is present and the amount is greatest at the regions where the most complete mixing of the river and of the sea water takes place. Evidently each water source contributes certain conditions or factors favorable for plankton growth. Further investigation to determine the exact nature of these conditions is in progress. (6) Mass movements of the epithalassa accompany tidal changes, resulting in variations of salinity, temperature and plankton at any point according to the source of the translocated water and the phase of the tide.

Introduction

The original problem which gave the primary impetus to the present investigation was the feasibility of diverting the migrations of salmon by closing Canoe Pass, the most southerly outlet of the Fraser River. This necessitated a general survey of the area and an estimate of the relative effect of this river in comparison with the other rivers entering the Strait of Georgia; a study of the effect of tides and other influences upon the salinity, temperature, pH, phosphate, silicate, nitrogen content, and plankton, including zooplankton and phytoplankton of the Strait of Georgia; and in turn the effect of physical and chemical conditions and the distribution of fish food upon marine organisms including fish and shellfish.

Fraser (7) and Cameron and Mounce (3) published accounts previously with reference to physical and chemical conditions at a number of regions, especially in the vicinity of the Pacific Biological Station, Departure Bay, Nanaimo.

[1] *Manuscript received March 23, 1931.*
Contribution from the Department of Botany, University of British Columbia, Vancouver, Canada, with financial assistance from the National Research Council of Canada.
[2] *Professor of Botany, University of British Columbia.*
[3] *Professor of Chemistry, Brandon College.*

Thompson (14), Gran and Thompson (8), Thompson and Van Cleve (16), Thompson and Wright (17) and Thompson, Miller, Hitchings and Todd (15) have been investigating the Puget Sound and San Juan region of neighboring waters with headquarters at the Puget Sound Biological Station, Friday Harbor.

At the Pacific Biological Station, Departure Bay, Nanaimo, Dr. Clemens, Director of the Station, has taken charge of a drift-bottle study of currents. Clemens (5, 6) and Williamson (19, 20) have published several papers on salmon migrations; Campbell (4), on Zooplankton; and Hutchinson, Lucas and McPhail (9, 10, 12, 13) on temperature, salinity, pH, silicate, phosphate and phytoplankton of the Strait of Georgia. The work is being continued by Carter, Fleming and Beal.

This account is limited to a consideration of the temperature, salinity, pH and phytoplankton of the Strait of Georgia and neighboring waters to a depth of 50 yards (45.7 metres) during the summer months.

Constant use has been made of Tide Tables of the Pacific Coast of Canada (1), British Admiralty Charts and the B.C. Pilot (2), and data have been obtained from Johnston's account of the sedimentation of the Fraser River delta (11).

Acknowledgment is made of numerous valuable articles on oceanographic conditions in other regions. A review of these does not fall within the scope of the present account, although conditions in other parts of the world have thrown much light upon the problem under consideration.

Methods and Measurements

The methods used are described in earlier papers (9, 10, 12, 13); these are in accord with the methods recommended by the "Conseil Permanent International pour l'exploration de la mer." The metric system is used except in the determination of distance where miles* and yards** are the units. The investigation was started when a yard meter only was available for the cable measurements and for the sake of uniformity this unit has been retained. Temperature is expressed as degrees centigrade; salinity as grams of total halide expressed as chloride per litre, and plankton as volumes of centrifuged plankton material from 100,000 volumes of sea water.

General Description of the Strait of Georgia

The Straight of Georgia, situated between the southern portion of Vancouver Island and the mainland of British Columbia and the State of Washington is a great basin 140 miles long and of width varying from 20 to 30 miles. It is connected with the Pacific Ocean by several narrow passes. Boundary Pass and Rosario Strait at the south open into the Strait of Juan de Fuca; and Discovery Pass at the north continues to Seymour Narrows and Johnston Strait (Chart 1). A double chain of elongated islands parallel the southerly portion of Vancouver Island to form Stuart and Trincomali Channels. Active, Porlier and Gabriola Passes, between these islands, furnish additional extremely

*1 mi. = 1.60 kilometres; **1 yard = 0.914 metres.

constricted outlets. Texada Island, 30 miles long, is situated opposite Jervis Inlet and is accompanied by a smaller island, Lasqueti, located near mid-channel. At the north a number of circuitous channels connect indirectly with Johnston Strait and the Pacific Ocean. One known as the Hole-in-the-Wall has a very descriptive name (Chart 1).

Tides

The tides are most unusual. The chief flood enters through Juan de Fuca Strait and continues to the north end of the Strait of Georgia, especially on the east side where its effect passes through Sutil Channel and to Toba and Bute Inlets; another flood enters through Johnston Strait and Discovery Pass at the north, and affects the northwest portion of the Strait of Georgia especially along the shore of Vancouver Island. The latter flood precedes the former by approximately two hours. They result in eddies and turbulence which is marked southward to Texada Island. The tide which enters the Strait of Juan de Fuca floods one hour longer through Rosario Strait on the Washington side than through Haro Strait, toward Vancouver Island. The result is a great swirl which affects the area from Boundary Pass to Boundary Bay. This swirl was examined on August 17, 1929. At slack tide, a calm area extended with its centre two miles off East Point, Saturna Island, for a distance of two miles in every direction and was delimited by a circle of turbulence easily discerned from the centre and traversed at its southerly and northerly extremities during our cruise. The region of marked turbulence apparently increases in diameter toward mid-tide until it reaches the Point Roberts-Boundary Pass line.

The remarkable phenomenon of a tidal current which persists for the complete 24 hr. in a single direction, southeastward, was observed July 18, 1929, in Tumbo Channel, three miles N.E. of East Point, Boundary Pass. Since this small channel, two miles long, opens directly at each end to the Strait of Georgia one would not expect tidal currents to be marked; at midtide, however, a sixty-foot boat at anchor was nearly capsized. Similarly the "B.C. Pilot" records that "close along the southeast side of East Point the stream always sets northeastward."

Tidal currents are increased in complexity by fiords such as Burrard, Jervis, Toba, Bute and Saanich Inlets and Howe Sound, which vary in length from 40 to 80 miles, attain a depth in some cases of 300 fathoms (548 m.) and are limited by shallow and often narrow mouths.

These unusual tidal conditions are superimposed upon the well-known characteristic of the tides of Juan de Fuca, Puget Sound and the Strait of Georgia, namely, the inequality of alternate daily tides; the one fluctuates in height by approximately twelve feet, the other by one-quarter of that amount (1, 2, B.C. Pilot, Tide Tables).

Rivers Flowing into the Strait of Georgia

The Fraser River has a minimal average annual discharge of 80 million acre-feet; one-fifth of this amount is discharged during the months of October to

April while 64 million acre-feet of water is contributed during the summer period, May to September (11, 12, 18).

It is estimated that this water would occupy the upper portion of the basin of the Strait of Georgia in the part most directly affected or northward to 49°25′ to a depth of 80 ft. if not removed. The Squamish River, during the same period, discharges on the average about one-twelfth that amount; the

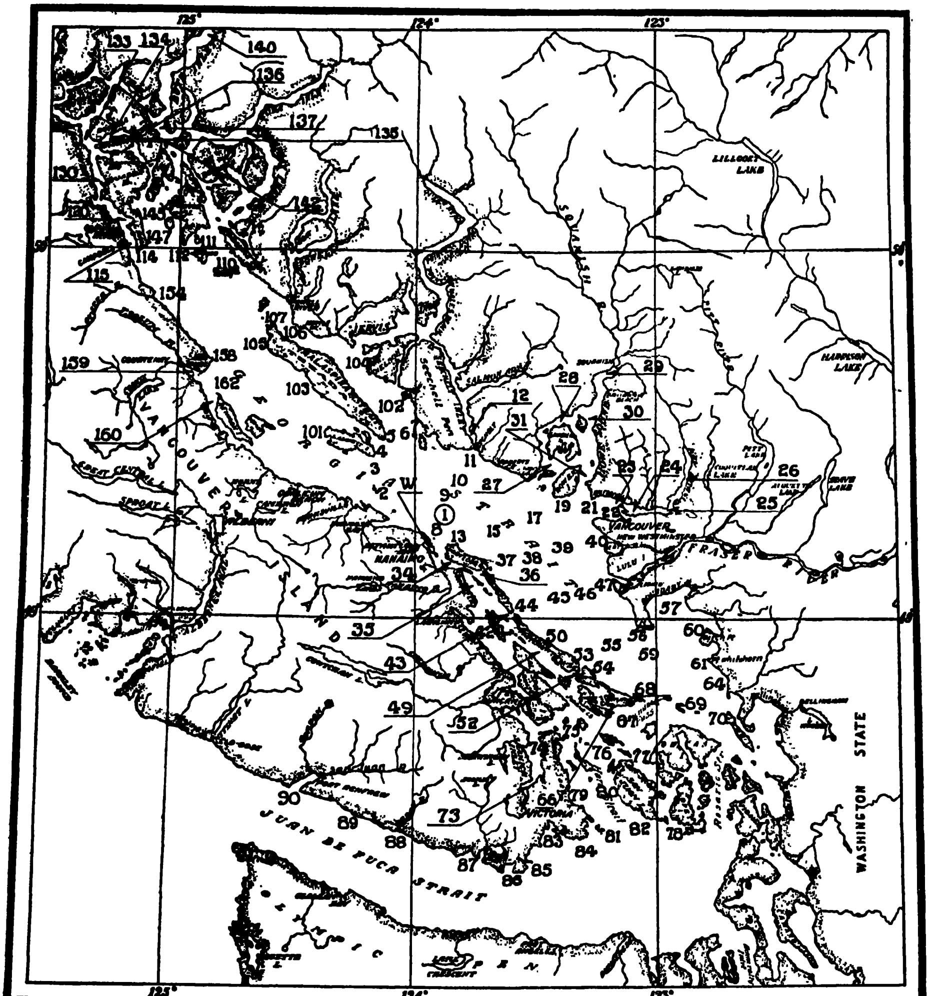


Chart 1. *Stations, Strait of Georgia.*

Nanaimo River less than one one-hundredth on the average; and a number of smaller rivers a proportion which has an effect on salinity of local significance only. The rivers flowing into Jervis, Bute and Toba Inlets, although relatively small and of undetermined discharge have a decided effect upon the waters not only within the limits of their respective inlets but also upon the neighboring regions of the Strait of Georgia.

TABLE I

SURFACE SALINITY, TEMPERATURE, PLANKTON AND pH ON LINE WESTWARD FROM THE FRASER RIVER MOUTH

Reference No.	Station	Location	Date	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
1	35	N. 49°6′, W. 123°47′	July 10, 1928	1:00 p.m.	E 1	18.8	12.33	8.55	45.0
2	35	Stuart Channel, Northern part	July 16, 1927	8:30 a.m.	E 1	19.30	13.58	—	17.5
3	35a		July 14, 1926	12:30 p.m.	E 5	19.9	14.50	8.5	41.0
4	35		Aug. 13, 1926	3:00 p.m.	F 3	17.9	15.12	8.50	36.0
5			July 29, 1926	11:15 a.m.	E 3	17.3	15.23	8.4	26.0
6	35a		July 29, 1926	9:10 a.m.	E 1	16.6	15.33	8.4	50.0
7	35b		July 29, 1926	10:40 a.m.	E 2	16.1	15.48	8.4	67.0
8	42a	N. 48°56′, W. 123°42′	July 14, 1926	1:30 p.m.	E 6	20.6	14.80	8.5	50.0
9	42	Stuart Channel S.	July 29, 1926	12:07 p.m.	E 4	17.20	15.38	—	—
10	35e	N. 49°5′, W.123°38′	June 25, 1927	2:50 p.m.	E 1	14.50	13.81	—	87.0
11	35d	Trincomali Ch. N.	June 16, 1927	2:30 p.m.		14.95	14.12	8.52	—
12	43b	N. 48°59′, W. 123°36′	July 11, 1928	6:30 p.m.	F 6	20.35	7.28	8.45	—
13	43	Near Porlier Pass	July 16, 1927	7:40 p.m.	F 6	13.60	13.77	—	32.5
14	43	Trincomali Ch.	June 25, 1927	2:50 p.m.	E 1	15.00	14.18	8.50	75.0
15	43a		July 12, 1928	8:00 a.m.	F 2	14.4	14.68	8.35	62.0
16	44b	N. 49°13′, W. 123°31′	July 11, 1928	6:10 p.m.	E 6	20.7	5.78	8.45	—
17	44	Near Porlier Pass	July 15, 1927	3:20 p.m.	F 3	19.20	6.17	8.40	1.2
18	44	Str. of Georgia	July 11, 1928	6:20 p.m.	F 1	20.4	6.52	8.42	—
19	44a		July 30, 1926	5:35 p.m.	F 3	19.0	6.83	8.35	8.4
20	44		July 16, 1926	4:20 p.m.	F 2	20.0	8.12	8.55	—
21	44		Aug. 6, 1926	4:05 p.m.	F 4	17.80	13.48	8.70	32.0
22	45	N. 49°4′, W. 123°29′	July 19, 1929	1:06 p.m.	F 3	17.75	4.74	7.88	0.7
23	45a	Str. of Georgia	July 11, 1928	5:20 p.m.	E 6	19.7	7.63	8.47	—
24	45	W. of mid-channel	July 11, 1928	5:45 p.m.	E 6	19.70	7.84	8.45	4.0
25	45c		July 28, 1928	10:11 p.m.	F 2	19.1	8.51	8.50	—
26			July 28, 1928	10:00 a.m.	F 2	18.1	12.09	8.64	—
27	45c		July 28, 1928	10:11 a.m.	F 2	18.2	12.28	8.67	—
28	45		Aug. 6, 1926	5:00 p.m.	F 5	18.20	12.52	8.65	32.0
29	46a	N. 49°5′, W. 123°24′	July 11, 1928	4:50 p.m.	E 5	19.10	1.81	8.25	0.2
30	46	Str. of Georgia	July 11, 1928	5:03 p.m.	E 6	20.1	2.83	8.25	0.2
31	46	E. of mid-channel	Aug. 6, 1926	6:00 p.m.	E 1	18.0	10.97	8.55	9.0
32	47	N. 49°6′, W. 123°20′	July 30, 1927	3:36 p.m.	F 3	18.48	0.115	8.02	1.2
33	47	Str. of Georgia	June 23, 1927	5:08 p.m.	E 5	15.0	0.29	7.80	0.7
34	47	Near lightship	July 11, 1928	4:36 p.m.	E 5	18.50	1.74	8.23	1.2
35	47		July 12, 1926	10:00 a.m.	E 3	17.4	4.17	8.5	—
36	47		Aug. 11, 1926	2:53 p.m.	F 2	18.30	4.86	8.30	1.2
37	47a		July 12, 1926	9.30 a.m.	E 2	17.0	8.72	8.45	6.0
38	47b		July 12, 1926	10:15 a.m.	E 3	16.9	11.33	8.5	—

The temperatures of these rivers, as they enter the Strait, vary greatly according to their sources and the time of the year. In January the Fraser River at New Westminster has a mean temperature of 4° C.; in April, 6.5 to 7.3° C.; in May, 7.3 to 10° C.; in June, 10 to 15° C.; in July, 15 to 17.5° C. and in August, 16 to 17.5° C. In contrast the surface temperature near the head of Howe Sound on July 9, 1927, was 9.5° C. and on August 6 it was 11.9° C. On September 19, 1928, the surface temperature near the entrance of Comox River was 16.6° C. The three rivers cited are typical of three classes; the first, those of great length which are snow-fed primarily but which become warmed by insolation on their way to the sea, such as the Fraser River; second, glacial streams with their sources near the coast, such as those flowing into Howe Sound and Jervis, Toba and Bute Inlets; and third, those which are relatively short and which are fed by mountain or upland lakes. The rivers on the eastern slope of Vancouver Island, namely Campbell, Oyster, Comox, Qualicum, Englishman, Nanaimo, Chemainus and Cowichan are included in this class. The primary effect upon the temperature of the Strait is evidently dependent upon the factors of volume and temperature.

Surface Salinity, Temperature, Plankton and pH on a Line West from the Fraser River

Stuart Channel

Stations 35 and 42 received water of high salinity from the Strait of Juan de Fuca through Sansum Narrows and Trincomali Channel to the south; sea water mixed with Fraser River water through Dodds Narrows and Porlier Pass and fresh water from the Chemainus River chiefly. The chlorinity shows a variation during July and August from 12.33 to 15.48 gm. per litre. The low values in early July are obviously the result of the flood water of the Chemainus River which occurs in May and June. The variation is not great either from month to month or with the tides; the mid-flood water on August 13 differed from the mid-ebb of July 29, by only 0.6 gm. Cl per litre. The temperature varied from 16.1 to 20.6° C. during this period and showed the evidence of a stable upper layer warmed by insolation. The plankton is consistently abundant and the pH high (8.4) as a result of the utilization of carbon dioxide by the phytoplankton.

Trincomali Channel

The variations in salinity and temperature are less than in Stuart Channel; for instance, there is little tidal variation (cf. 14.18 gm. Cl for the ebb and 13.77 for the flood). The salinities are intermediate between the extremes of Stuart Channel while the temperatures are uniformly lower, 13.7 to 14.6° C. This water is subject to marked tidal currents and the resulting turbulence. The two substations 43 (a) and 43 (b), situated a mile apart, the former in Trincomali Channel and the latter in the adjacent waters of the Strait of Georgia outside Porlier Pass, show a decided difference in both temperature and salinity (14.4° compared with 20.35° C. and 14.68 gm. Cl compared with 7.28 gm. Cl).

The evidence points to the barrier effect of passes on the escape of surface fresh water from the Strait of Georgia. The phytoplankton quantity and the pH are similar to those of Stuart Channel.

Strait of Georgia

The surface samples on a line across the Strait of Georgia from Porlier Pass to the lightship at the mouth of the Fraser River show a gradation from a minimal chlorinity of 5.78 gm. Cl per litre at Station 44 (Table 1) on the ebb tide to 0.29 at station 47. This tide carries the Fraser River water, which was taken northwestward by the flood, southward again to the westerly stations. The chlorinity values for the flood are affected by the sea water entering through the Straits and had maximum values during July and August of 13.5 gm. Cl at station 44 and 4.9 gm. Cl at station 47 (Table I). A very sudden change in salinity occurs within two miles of the lightship. On July 12, 1926, at 10:00 a.m. a sample was taken: chlorinity, 4.17; temperature, 17.4° C. Fifteen minutes later, less than two miles distant the values were: chlorinity, 11.33 gm. Cl; and temperature, 16 9° C. Lines of separation between the turbid water from the Fraser River and the blue water of the Strait are often very evident, and as previously recorded several similar lines may be observed frequently, forming arcs the radii of which are greatest northwestward from the Fraser River mouth.

The temperature of the surface water does not vary entirely with chlorinity along the Porlier Pass to the lightship line. On the ebb it was highest, 20.7° C., at the most distant station, that is, at 44, and decreased to 17.5° C., the usual summer temperature of the Fraser River, as the lightship was approached. The water carried northwestward by the flood tide and finally southward to station 44 by the ebb becomes warmed by insolation. The lower temperature near the river mouth is further shown in June; for instance, on June 23, 1927 the chlorinity was 0.29 and the temperature, 15.0° C. The flood tide on the other hand was characterized during July and August by a higher temperature at the lightship (*e.g.*, 15 06 and 15.08° C.) than at more distant points, especially at the last of this phase (for instance, 13.62° C. at station 46, Aug. 6, 1926). The colder water from Juan de Fuca is increased in temperature on the flood tide in proportion to its degree of mixing with the warmer Fraser River water as well as by solar heating and consequently time and distance become factors.

The surface plankton at the lightship is scarcely measurable and the pH is correspondingly low (*e.g.*, 7.8 and 8.0) at station 46, two and one-half miles distant, and the greatest recorded surface phytoplankton quantity is 9.0 units with a pH of 8.55; and at stations 45 and 44, *i.e.*, at distances of five and seven miles the plankton had increased to 32.0 units at the optimum conditions. The pH at these points, respectively, was 8.65 and 8.70. The greatest plankton abundance occurs at a chlorinity 12.5 to 13.5 gm. Cl per litre north of the Fraser River; that is, where the mixing of river and sea water is most complete. The very high pH at these points is explained by two considerations: (1) the rapid photosynthesis carried on by the abundant phytoplankton in the more

TABLE II

TEMPERATURE, SALINITY, PH AND PLANKTON AT 10 YARDS (9.14 METRES) ON A LINE WESTWARD FROM THE FRASER RIVER MOUT

Reference No.	Station	Location	Date	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
39	35e	N. 49°6′, W. 123°46′	July 10, 1928	2:54 p.m.	E 2	13.13	14.70	8.25	22.5
40	35b	Stuart Channel N.	Aug. 2, 1928	10:22 a.m.	E 3	13.57	—	8.20	—
41	35		July 29, 1927	10:44 a.m.	E 5	13.84	14.91	8.23	5.80
42	35		July 29, 1926	10:24 a.m.	E 2	12.10	16.19	—	18.5
43	42	N. 48°50′, W. 123°42′							
		Stuart Channel S.	July 29, 1926	11:45 a.m.	E 3	12.58	16.20	7.95	1.3
44	43	N.48° 59′, W. 123°36′	Aug. 6, 1926	8:44 a.m.	E 3	15.18	14.61	8.35	18.0
45	43	Trincomali Ch. N.	July 29, 1927	1:32 p.m.	F 2	11.32	15.24	8.25	55.0
46	44	N. 49°2′, W. 123° 31′	July 30, 1927	5:56 p.m.	F 5	16.42	13.89	8.62	22.5
47	44	Near Porlier Pass	Aug. 6, 1926	3:56 p.m.	F 3	17.15	14.41	8.30	—
48	44	Str. of Georgia	July 16, 1926	4:15 p.m.	F 2	13.90	15.87	8.30	—
49	45	N. 49°3′, W. 123°27′							
		Str. of Georgia	Aug. 6, 1926	4:45 p.m.	F 4	16.45	14.74	8.50	8.0
50	45	W. of mid-channel	July 11, 1928	5:32 p.m.	E 6	13.67	14.77	8.30	12.5
51	46	N. 49°5′, W. 123°24′	Aug. 6, 1926	5:50 p.m.	F 6	13.62	15.78	8.20	—
52	47	N. 49°6′, W. 123°20′	July 11, 1928	4:18 p.m.	E 5	14.14	14.46	8.39	22.5
53	47	Near lightship	Aug. 11, 1926	2:31 p.m.	F 2	16.62	15.06	8.40	10.0
54	47	Str. of Georgia	July 30, 1927	3:14 p.m.	F 3	13.43	15.08	8.28	27.5
55	47a		Aug. 12, 1926	9:16 a.m.	E 2	16.15	15.12	8.45	—

TABLE III

TEMPERATURE, SALINITY, PH AND PLANKTON, AT 6 YARDS (5.5 METRES) ON A LINE WESTWARD FROM THE FRASER RIVER MOUTH

Reference No.	Station	Location	Date	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
56	35	N. 49°6′, W. 123°46′	July 29, 1927	10:49 a.m.	E 5	14.45	14.78	8.38	81.9
57	35a	Stuart Ch. N.	July 29, 1926	10:28 a.m.	E 2	12.75	16.32	8.10	—
58	42a	N. 48°56′, W. 123°42′	July 14, 1926	2:35 p.m.	F 1	18.65	14.91	8.5	—
59	42	Stuart Ch., S.	July 29, 1926	11:58 a.m.	E 3	13.12	15.82	—	5.0
60	43	N. 48°59′, W. 123°36′	Aug. 6, 1926	8:45 a.m.	E 3	15.50	14.62	8.85	20.0
61	43	Trincomali Ch. N.	July 29, 1927	1:37 p.m.	F 2	14.33	14.74	8.30	70.0
62	44	N. 49°2′, W. 123°31′	July 30, 1927	6:00 p.m.	F 5	17.23	13.27	8.90	100.0
63	44	Str. of Georgia	Aug. 6, 1926	4:00 p.m.	F 3	17.68	13.78	8.50	—
64	45	N. 49°3′, W. 123°27′	July 11, 1928	5:36 p.m.	E 6	14.80	14.20	8.42	45.0
65	45	Str. of Georgia	Aug. 6, 1926	4:49 p.m.	F 4	17.02	14.38	8.50	24.0
66	46	N. 49°5′, W. 123°24′	Aug. 6, 1926	5:58 p.m.	F 6	16.09	14.71	8.40	—
67	47	N. 49°6′, W. 123°20′	July 11, 1928	4:27 p.m.	E 5	17.64	10.57	8.42	30.0
68	47	Near lightship	July 30, 1927	3:18 p.m.	F 3	16.20	14.02	8.06	1.46
69	47a	Str. of Georgia	Aug. 12, 1926	9:20 a.m.	E 2	16.96	14.57	8.45	—
70	47		Aug. 11, 1926	2:35 p.m.	F 2	17.22	14.73	8.40	22.0

TABLE IV

SURFACE SALINITY, TEMPERATURE, pH AND PLANKTON ON A MID-CHANNEL LINE THROUGH HARO STRAIT, STRAIT OF GEORGIA AND DISCOVERY PASSAGE

Reference No.	Station	Location	Date	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
71	82a	N. 48°27′, W. 123°3′	July 15, 1926	4:54 p.m.	F 4	13.00	16.98	8.15	8.0
72	80	Haro Strait	July 15, 1926	5:40 p.m.	F 6	13.00	16.52	8.25	35.0
73	80	N. 48°32′, W. 123°12′	July 15, 1926	6:35 p.m.	E 1	11.95	17.35	8.05	17.0
74	77	Haro Strait	Aug. 17, 1929	11:45 a.m.	F 3	12.70	16.59	7.82	1.5
75	77	N. 48°37′, W. 123°4′	Aug. 16, 1929	2:33 p.m.	F 4	13.60	16.60	7.95	0.5
76	77a		July 15, 1926	2:55 p.m.	F 3	13.50	16.77	8.10	3.6
77	77a		July 15, 1926	11:50 a.m.	E 3	13.00	16.78	8.05	3.6
78	77		July 15, 1926	12:20 p.m.	E 4	13.20	16.79	8.10	7.2
79	77		Aug. 17, 1929	10:45 a.m.	F 2	11.70	17.25	7.80	1.5
80	76	Haro Strait N. 48°38′, W.123°15′	July 15, 1926	10:10 a.m.	E 2	13.40	16.42	8.15	35.0
81	76		June 24, 1927	6:08 p.m.	E 5	11.00	16.42	8.20	25.0
82	68b	Boundary Pass	July 27, 1928	2:00 a.m.	E 3	17.00	13.22	8.62	50.0
83	68a	N.48°46′, W. 123°3′	July 15, 1928	2:42 p.m.	E 1	14.00	13.84	—	17.5
84	68		July 13, 1928	1:28 p.m.	F 5	14.20	14.00	8.22	16.2
85	68b		July 26, 1928	12:30 p.m.	E 3	15.10	14.43	8.40	7.50
86	68b		July 26, 1928	11:21 a.m.	E 2	16.30	14.49	8.40	50.0
87	68	Boundary Pass	Aug. 13, 1926	8:00 a.m.	F 6	12.10	16.38	8.25	17.0
88	68	N. 48°46′, W. 123°3′	July 25, 1927	8:51 a.m.	F 1	10.02	16.64	8.08	20.0
89	68s3		Aug. 17, 1929	12:20 p.m.	F 2	13.10	16.30	7.90	1.0
90	68s2		Aug. 17, 1929	12:25 p.m.	F 2	12.70	16.49	7.90	2.0
91	68s1		Aug. 17, 1929	12:30 p.m.	F 2	12.90	16.44	7.90	2.0
92	68		Aug. 17, 1929	12:45 p.m.	F 2	15.20	15.32	8.02	1.5
93	68n1		Aug. 17, 1929	12:50 p.m.	F 2	17.20	13.37	8.25	1.5
94	68n3		Aug. 17, 1929	1:02 p.m.	F 3	18.90	12.05	8.30	1.5
95	68n4		Aug. 17, 1929	1:18 p.m.	F 3	18.80	11.40	8.40	1.0
96	68		Aug. 17, 1929	1:35 p.m.	F 3	19.30	12.18	8.40	0.5
97	57	S.E. Active Pass	July 29, 1927	6:23 p.m.	F 6	19.46	8.88	8.70	1.5
98	54	Strait of Georgia	July 19, 1929	11:45 a.m.	F 2	16.93	11.05	8.10	0.7
99	54	N. 48°54′, W. 123°15′	July 30, 1926	4:05 p.m.	F 2	17.20	12.62	8.35	28.0
100	54		July 17, 1929	11:00 a.m.	F 3	16.24	13.79	8.60	2.0
101	54		July 19, 1929	11:45 a.m.	F 2	16.93	11.05	8.10	0.7
102	54		July 18, 1929	8:15 a.m.	F 1	15.00	14.96	7.98	0.4

103	55	Strait of Georgia	Aug. 12, 1926	1:10 p.m.	E 6	17.00	13.54	8.50	64.0
.04	55	N. 48°56′, W. 123°11′	July 28, 1928	8:15 a.m.	E 6	17.00	13.74	8.70	—
.05	55(5)		July 28, 1928	8:30 a.m.	E 6	16.9	13.96	8.65	—
.06			July 28, 1928	8:45 a.m.	E 6	16.2	14.15	8.66	—
.07	55(5)		July 28, 1928	7:55 a.m.	E 6	15.90	14.55	8.47	60.0
.08	53	N. 48°55′, W. 123°16′	July 19, 1929	12:02 p.m.	F 2	17.18	11.60	8.05	—
.09	53	Strait of Georgia	July 16, 1926	2:20 p.m.	F 1	19.8	12.03	8.60	0.7
.10	53a	N.E. of Active Pass	Aug. 11, 1926	6:25 a.m.	F 6	16.6	12.89	8.50	—
.11	50	Strait of Georgia	July 15, 1927	4:20 p.m.	F 4	19.10	5.27	8.65	0.5
112	50a	E. of Galiano Island	Aug. 11, 1926	4:10 p.m.	F 4	—	6.57	8.50	20.0
113	50	N. 48°58′, W. 123°23′	July 30, 1926	4:50 p.m.	F 2	17.80	8.50	8.35	27.0
114	50b		Aug. 12, 1926	7:00 a.m.	F 6	16.8	8.66	8.45	—
115	50		July 19, 1929	12:15 p.m.	F 3	17.25	10.01	8.22	—
116	50		July 16, 1926	3:30 p.m.	F 1	19.5	11.08	8.55	0.7
117	50b		Aug. 11, 1926	4:35 p.m.	F 4	18.	11.67	—	40.0
118	50		Aug. 17, 1929	4:00 p.m.	F 5	19.10	14.45	8.40	0.5
119	45c	Strait of Georgia	July 28, 1928	10:15 a.m.	F 2	20.0	1.15	8.03	—
120	45a	5 miles N.E. Porlier P.	July 11, 1928	5:20 p.m.	E 6	19.7	7.3	8.47	—
121	45	N. 49°4′, W. 123°27′	July 11, 1928	5:45 p.m.	E 6	19.70	7.84	8.45	4.0
122	45c		July 28, 1928	10:12 a.m.	F 2	19.1	8.51	8.50	—
123	45c		July 28, 1928	10:10 a.m.	F 2	18.2	12.28	8.67	—
124	45		Aug. 6, 1926	5:00 p.m.	F 5	18.20	12.52	8.65	32.0
125	46a	Op. Fraser River	July 11, 1928	4:50 p.m.	E 6	19.10	1.81	8.25	—
126	46	Strait of Georgia	July 11, 1928	5:03 p.m.	E 6	20.1	2.83	8.25	—
127	46	N. 49°5′, W. 123°24′	Aug. 6, 1926	6:00 p.m.	E 1	18.0	10.97	8.55	1.90
128	40a	Strait of Georgia	June 23, 1927	3:40 p.m.	E 3	17.6	1.25	7.85	0.5
129	40	N. 49°11′, W. 123°20′	June 23, 1927	4:12 p.m.	E 4	16.2	1.38	8.00	—
130	40	N.W. Fraser River	Aug. 11, 1926	1:28 p.m.	E 6	20.0	2.64	8.00	2.4
131	40a		Aug. 11, 1926	1:43 p.m.	F 1	20.5	3.26	8.10	—
132	37	Strait of Georgia	July 28, 1928	10:15 p.m.	F 2	20.0	1.15	8.03	—
133	37	E. of Gabriola Pass	July 28, 1928	10:30 p.m.	F 2	19.5	5.78	8.50	—
134	37	N. 49°9′, W. 123°33′	July 28, 1928	10:44 p.m.	F 2	19.0	7.70	8.58	—
135	14	Strait of Georgia	July 4, 1927	9:20 a.m.	E 2	15.80	9.75	8.38	5.6
136	14a	N.W. Entrance Island	July 11, 1928	11:31 a.m.	F 3	19.4	9.90	8.42	—
137	14	N. 49°13′, W. 123°25′	July 11, 1928	11:05 a.m.	F 3	19.5	10.23	8.50	30.0
138	14		July 12, 1927	11:15 a.m.	E 3	17.75	12.94	8.40	5.0
139	14		Aug. 10, 1926	11:58 a.m.	E 6	18.30	13.68	8.80	48.0
140	14		July 19, 1929	2:15 p.m.	F 4	15.96	14.50	8.22	—
141	14		Aug. 17, 1929	7:25 p.m.	E 1	19.40	14.53	8.25	4.0

TABLE IV—*Continued*

Reference No.	Station	Location	Date	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
142	15a	Strait of Georgia	July 11, 1927	11:53 a.m.	F 5	19.7	8.98	8.45	—
143	15	N.E. Entrance Island	July 11, 1927	11:42 a.m.	F 4	19.5	10.24	8.42	20.0
144	15	N. 49°14′, W. 123°21′	July 4, 1927	9·40 a.m.	E 2	15.70	10.92	8.38	7.5
145	15		July 6, 1927	4:55 p.m.	F 4	17.2	11.18	—	6.5
146			July 26, 1929	12:15 p.m.	E 4	17.10	13.89	8.30	—
147	16	Strait of Georgia	July 11, 1928	12:14 p.m.	F 5	20.1	8.90	8.52	12.5
148	16	N. 49°14.5′, W. 123°37′	July 26, 1929	1:02 p.m.	E 5	17.40	12.30	8.20	—
149	9	N. 49°21′, W. 123°50′	Aug. 4, 1926	4:36 p.m.	F 6	20.00	12.01	8.90	60.0
150	10	N. 49°23′, W. 123°48′	Aug. 4, 1926	4:00 p.m.	F 6	19.00	12.61	8.85	65.0
151	1	Strait of Georgia	June 21, 1927	11:34 a.m.	E 2	18.60	8.08	8.30	0.5
152	1	N. 49°19′, W. 123°52′	Aug. 1, 1928	12:07 p.m.	F 1	18.80	9.70	8.70	1.0
153	1		Aug. 4, 1926	5:35 p.m.	E 1	19.0	12.18	8.60	60.0
154	1		Aug. 18, 1927	8:21 a.m.	F 5	20.30	12.57	8.65	22.5
155	1		Oct. 21, 1926	—	—	11.38	13.96	8.10	—
156	1		July 10, 1929	11:45 a.m.	E 4	14.89	14.64	8.6	1.0
157	1		June 29, 1929	11:23 a.m.	E 2	15.40	14.65	8.5	1.0
158	1		Sept. 20, 1928	1:39 p.m.	E 2	13.60	15.03	8.2	—
159	1		Dec. 18, 1928	—	—	6.79	15.64	—	—
160	1		Oct. 23, 1928	—	—	10.04	15.92	8.1	—
161	1		Mar. 31, 1927	—	—	7.8	16.24	7.8	—
162	1		Feb. 3, 1927	—	—	7.2	16.41	8.0	—
163	1		Feb. 9, 1927	11:50 a.m.	E 6	7.20	16.58	7.98	—
164	1		Apr. 25, 1929	11:23 a.m.	E 2	15.40	14.65	8.5	1.0
165	1		Mar. 24, 1929	12:15 p.m.	F 1	8.32	16.84	7.85	0.2
166	1		Mar. 2, 1929	11:00 a.m.	E 2	7.20	16.97	7.75	0.4
167	2e	N. 49°20′, W. 124°9′	June 27, 1927	12:30 p.m.	F 3	16.90	10.31	8.30	15.0
168	2e		June 27, 1927	12:00 p.m.	F 2	15.80	11.52	8.28	21.2
169	2b		June 28, 1927	8:10 p.m.	E 2	16.60	11.82	8.45	8.2
170	2	Strait of Georgia	Aug. 11, 1928	9:35 a.m.	F 2	17.8	12.12	8.45	2.5
171	2	Off Ballenas Island	July 30, 1926	11:22 a.m.	F 3	18.00	12.82	8.55	58.0
172	2a	N. 49°20′, W. 124°9′	July 30, 1926	2:40 p.m.	F 6	17.97	13.28	8.55	38.0
173	2b		July 23, 1926	6:40 a.m.	E 6	16.85	13.92	8.55	0.5
174	2W		Aug. 8, 1929	4:00 p.m.	E 3	18.00	14.64	8.30	1.5
175	2		Aug. 6, 1928	12:00 p.m.	E 2	15.4	14.90	8.48	4.0

176	3	Strait of Georgia	June 28, 1927	7:10 p.m.	E 1	16.20	13.50	8.40	12.5
177	3	S. Lasqueti Island	Aug. 6, 1928	1:00 p.m.	E 4	17.6	13.67	8.57	0.5
178	3	N. 49°23′, W. 124°11′	July 30, 1926	4:50 p.m.	E 1	17.0	14.45	8.45	6.2
179	3w		Aug. 8, 1929	6:00 p.m.	E 5	18.80	15.49	8.30	1.0
180	101	Strait of Georgia	June 28, 1927	5:25 p.m.	F 6	16.35	12.44	8.40	7.5
181	101s	N.W. Lasqueti Island	Aug. 11, 1928	8:45 a.m.	F 1	17.5	12.90	8.43	0.5
182	101s	N. 49°35′, W. 124°30′	Aug. 6, 1928	1:50 p.m.	E 5	17.7	13.82	8.50	2.5
183	101n		Aug. 11, 1928	7:35 a.m.	E 6	16.1	14.44	8.22	5.0
184	101n	N. 49°35′, W.124°30′	Aug. 6, 1928	2:45 p.m.	F 1	16.8	14.80	8.47	2.5
185	101w		Aug. 8, 1929	7:00 p.m.	E 5	19.80	15.33	8.30	3.0
186	103	W. Texada Island	June 28, 1927	3:10 p.m.	F 4	14.90	14.80	8.28	7.5
187	103	N. 49°40′, W. 124°35′	Aug. 6, 1928	3:25 p.m.	F 1	16.3	14.99	8.45	2.5
188	105	N.W. Texada Island	Aug. 6, 1928	6:15 p.m.	F 4	18.3	13.36	8.67	4.5
189	105	N. 49°45′, W.124°42′	June 28, 1927	1:12 p.m.	F 3	16.70	13.80	8.52	2.5
190	162		Aug. 10, 1928	12:40 p.m.	F 5	18.4	13.04	8.40	2.5
191	162		Aug. 9, 1929	11:57 a.m.	E 4	16.74	15.55	8.40	0.5
192	154		Aug. 10, 1928	11:30 a.m.	F 4	17.7	13.68	—	16.0
193	154		Aug. 9, 1929	2:50 p.m.	F 1	16.21	15.75	8.42	1.5
194	153		Aug. 9, 1928	6:20 p.m.	F 1	16.9	15.0	—	1.0
195	152		Aug. 9, 1928	6:05 p.m.	F 1	18.2	14.74	—	—
196	151		Aug. 9, 1928	5:50 p.m.	F 1	18.1	14.25	—	—
197	150		Aug. 9, 1928	5:35 p.m.	F 1	19.1	14.42	—	—
198	149		Aug. 9, 1928	5:20 p.m.	E 6	19.9	12.83	—	5.0
199	114	N. 49°59′, W. 125°10′	Aug. 7, 1928	10:05 a.m.	E 1	18.0	13.55	8.60	2.5
200	115	N. 50°0′, W. 125°13′	Aug. 6, 1929	10:25 a.m.	E 1	17.8	13.56	8.55	4.0
201	115		Aug. 9, 1929	5:49 p.m.	F 3	15.20	16.00	8.32	5.0
202	116		Aug. 7, 1928	10:35 a.m.	E 1	17.7	13.76	8.60	4.0
203	120	Discovery Passage Quathiaski Cove	Aug. 8, 1928	5:00 a.m.	F 2	14.4	15.17	—	12.5
204	120	N. 50°1′, W. 125°22′	Aug. 7, 1928	1:00 p.m.	E 4	13.0	15.77	8.27	5.0
205	120		Aug. 7, 1928	7:01 p.m.	F 3	12.6	15.93	8.22	6.2
206	120		Aug. 7, 1928	9:00 p.m.	F 5	11.3	16.30	8.00	7.5
207	120n.1.		Aug. 10, 1929	8:00 a.m.	F 6	10.54	16.71	7.78	0.5
208	130	Discovery Passage Plumper Bay	Aug. 8, 1928	11:20 a.m.	E 1	12.0	16.04	8.10	0.5
209	130	N. 50°10′, W. 125°22′	Aug. 8, 1928	4:30 p.m.	F 1	11.2	16.34	8.08	5.0
210	133	N. 50°17′, W. 125°27′	Aug. 9, 1928	9:40 a.m.	F 5	11.8	16.16	8.13	2.5
211	133		Aug. 10, 1929	11:41 a.m.	E 2	10.45	16.79	7.80	0.5

intense light near the surface and the consequent lowering of the carbon dioxide content and, (2) the relatively small buffer action of these diluted waters because of the lower concentration, particularly of bicarbonates.

Temperature, Salinity, pH and Plankton at 10 Yards (9.14 Metres) Depth on a Line Westward from the Fraser River Mouth

The salinity at 10 yards (9.14 metres) shows much less variation either with distance from the river or as a result of tides. In all cases there is evidence of rather complete mixing of river and sea water; the lowest recorded chlorine values are about 14.0 and occurred on the late flood at station 44. At this distance of seven miles the effect of the fresh water is shown at a greater depth. This fact is emphasized by the relatively high salinity at a depth of 10 yards at the Fraser River mouth (average Cl = 15+). In Trincomali Channel there is a greater variation at 10 yards than in Stuart Channel. The reverse of this is the case at the surface. Apparently more water enters Porlier Pass from the 10 yards than from the surface level. This furnishes further evidence for the conservation of the fresh water in the Strait of Georgia (cf. 9, 10, 12).

The temperature is relatively uniform in Stuart Channel at 10 yards as compared with Trincomali Channel; summer records show the extremes of 12.12 and 13.84° C. in the former and 11.32 and 15.18° C. in the latter. The relatively high salinity at the lightship is accompanied by a somewhat lower temperature, but the difference in temperature is proportionately less since the water from the region of Point Roberts has been considerably warmed by radiation.

The plankton quantity is also much more uniformly high at the 10 yards level, a circumstance which is apparently related to the high degree of mixing. The pH is considerably lower even with a high plankton content. At station 43 with a plankton record of 55.0 units the pH is 8.25. At the greater depth photosynthesis is less rapid and the buffer action is greater due to the higher concentration of bicarbonates.

The values at six yards are intermediate and will be discussed under the description of Fig. 17 and 18.

Surface Salinity, Temperature, pH and Plankton on a Mid-channel Line Through Haro Strait, Strait of Georgia and Discovery Passage

Haro Strait

In Haro Strait the surface salinity during the summer ordinarily varies less than a gram per litre. On July 15, 1926, samples were taken before and after the passing of a tide-rip; the values were, before the eddy, chlorinity 16.52 gm. Cl per litre and temperature 13.00° C.; after the eddy, chlorinity 17.35 gm. Cl per litre and temperature 11.95° C. The column of water as a whole shows relatively little change in temperature and salinity, in fact the surface salinity after the eddy was somewhat higher than that at 50 yards, before the eddy. It would appear that the column is very unstable and readily inverted by

tidal currents. A similar result from the turbulence of a water column of nearly uniform density is shown for station 77. The greatest variations recorded were for two points, one in an open area and the other in a narrow passage (ref. No. 74, 79). On July 15, 1926, the chlorinities were the same, 16.42 gm., while the temperatures were 13.40° C. and 11.00° C., respectively. The samples were collected exactly an hour apart. Temperature increases to some extent with increased insolation but much less than in areas where there is a stable epithalassa.

Ordinarily there is a small quantity of phytoplankton on the flood tide coming from Juan de Fuca (0.5 to 3.6 units), while on the ebb the currents carry material from the Sidney and Swanson channels to give values as high as 35.0 units. The pH on the flood varies from 7.8 to 8.1 and may attain 8.25 on the ebb when the phytoplankton is abundant. Again high salinity is accompanied by considerable buffer action and a relatively stabilized pH.

Boundary Pass

The dividing line between the area of sea water dominance and that diluted by the river occurs at or near East Point, Boundary Pass; there is a change of position of several miles according to the phase of the tide, however. According to the authors' records the summer chlorinity range at station 68 was 13.22 to 16.64 gm. Cl and the corresponding temperatures were 17.0° C. and 10.02° C., the first reading in each case being on the mid-ebb, the latter on the mid-flood, July 27, 1928, and July 25, 1927, respectively. A series of samples collected over a distance of five miles, two miles south of East Point to three miles northwest, on August 17, 1929, showed a chlorinity change from 16.49 to 11.40 gm. Cl and temperatures from 12.70° C. to 18.80° C. and in a distance of 200 yards when opposite East Point the changes were 16.44 to 15.32 gm. Cl and 12.90° C. to 15.20° C.

The ebb waters whose chlorinity shows evidences of mixing are characterized by high plankton content (20 units). The end of the recorded flood period also brings phytoplankton from the Boundary Bay region because of the nature of the tidal swirl described above (Table IV, 84, 87). The early flood, however, has a low plankton value (0.5 to 2.0 units). Again, the highest pH, 8.62, was obtained for the water with most abundant phytoplankton, while on the flood tide the pH readings were repeatedly 7.90.

The Strait of Georgia, Southern Portion

A comparison of the salinities at stations 54 and 55 gives further evidence of the effect of the tidal swirl in Boundary Bay. Although station 55 is several miles nearer the Fraser River the salinity is generally higher (13.54 to 14.55 gm. Cl); there is a lower temperature (17.00 to 15.90° C.) and high plankton (60.0) and pH values (8.50 to 8.70) on the ebb, while at station 54 the flood, which might be expected to give high salinity values and low temperature, gives values which are relatively the reverse (Table IV, 98-104). Station 55 is within the Boundary Bay swirl while station 54 is westward beyond its immediate influence and within the area of the more direct influence of the Fraser River.

Station 53 is situated approximately the same distance from Active Pass as station 54; the former in a northeasterly direction, the latter southeasterly. The maximum temperature recorded for station 54 is 17.20° C. and for station 53 is 19.8° C.; similarly the chlorinity for the former is 14.96 gm. Cl and for the latter 12.89 gm. Cl. It would appear that the more saline water of lower temperature emerging with the flood through Active Pass is carried somewhat southward. This is in accord with the observation that the ebb tide is stronger on the western side of the Strait while the flood tide is stronger on the eastern side. On Aug. 17, 1927, more direct evidence was obtained from a series of surface samples collected on the flood tide along the east side of Saturna, Mayne, Galiano, Valdes and Gabriola Islands. Opposite the mid-point of Mayne Island the temperature was 19.30° C., the chlorinity 12.72 gm. Cl; at station 54 the readings were 18.73° C. and 13.76 gm. Cl; at station 53, 20.40° C. and 13.80 gm. Cl. A similar effect was observed opposite Porlier and Gabriola Passes.

Westward from the Fraser River

Remarkably low salinities are found on the ebb tide on a line westward from the Fraser River mouth and extending across the Strait of Georgia practically to Gabriola and Valdes Islands. At station 45 which is five miles from the lightship at the margin of the delta a reading of 1.15 gm. Cl was obtained near the beginning of the flood (Table IV, 119). The early flood entering along the east side forces the river water before it; a similar effect is seen at station 50 (Table IV, 111). The maximum summer chlorinity observed at this station 45 was 12.52 gm. Cl near the end of the ebb tide (Table IV, 124).

At station 40, near the north outlet of the Fraser River the lowest salinity was shown at the mid-ebb; at station 46 it occurred at the end of the ebb and at station 50 at the beginning of the flood; that is, the time factor enters since the more distant points are affected later as indicated by the phase of the tide.

The surface temperatures during July and August show consistently high values between 18.0 and 21° C. westward from the river. At station 45 chlorinity variations of 10.37 gm. Cl were recorded while the temperature variation was not more than 1.80° C.

The surface plankton is very low where the chlorinity is less than 12.0 gm. Cl. However, relatively high plankton values are found associated with chlorinities only slightly higher, for instance:— at station 45 at the end of the flood, when the chlorinity was 12.52, a sample containing 32 plankton units was collected.

The pH is uniformly high with abundant plankton (8.4-8.8) and in a number of cases it may be as high as 8.55, (Table IV, 127), with low plankton content. In such instances, however, it is found that there is high phytoplankton content at depths of two and four yards and the diffusion of carbon dioxide to these regions and wind action may account in part for this seeming anomaly.

North of the Fraser River

The effect of the river on both temperature and salinity is greater on the east side of the strait and it diminishes rapidly from mid-channel westward on a line

from Burrard Inlet to Nanaimo; the maximum chlorinity recorded at station 16 is 12.30 gm. Cl and the minimum 8.90 gm Cl, while five miles west at station 14 the extremes are 14.53 and 9.75 gm. Cl. The highest temperature at station 16 was 20.1° C. and at station 14 it was 19.5° C. As mentioned above the temperature increases with distance from the Fraser River since time is a factor in insolation, but this effect is overcome when the epithalassa is disturbed by mixing as a result of tidal or wind movements.

Farther north, the effect of the Fraser is carried farther westward as shown by the salinity at station 1, which is more than 25 miles northwest of the Fraser River mouth and which showed a minimal chlorinity in June 1927 of 8.08 gm. Cl and a maximal temperature in August 1927 of 20.30° C. (Fig. 8). The effect is most marked at the last of the flood or the beginning of the ebb.

The maximum phytoplankton is found at the last of the flood also, and occurs at the surface (20-65 units). One of the highest pH values was obtained at station 10 on Aug. 4, 1926, at the last phase of the flood tide, namely, pH 8.85, with plankton, 65 units. Frequently the change in plankton quantity is sudden, especially near the limits of an area of abundance. At station 2a, Ballenac Islands, on August 3, 1926, the phytoplankton changed from 19.0 to 38.0 units during a half-hour, shortly before the high water slack. A very small decrease in chlorinity from 13.48 to 13.28 gm. per litre was recorded.

The seasonal variation at station 1 is indicated in Table I, 155-168. The direct effect of atmospheric temperature and radiation on the surface water temperature is apparent and the high winter salinity may be noted also. These data are discussed in earlier papers (9, 12) and graphs are given to illustrate seasonal changes.

The Texada Island Region

At station 3, off the southern end of Lasqueti Island, the salinity varied from 13 to 15.50 gm. Cl. The epithalassa is however of sufficiently low density to be fairly stable and consequently the temperature is maintained during the summer at 16.2 to 18.8° C. (Table IV, 176-179). Station 101 had a similar temperature and somewhat higher chlorinity while at station 103, off the mid-point of Texada Island the chlorinity was relatively uniform, 14 80-14.49 gm. Cl, and the temperature was lower, 14.90 to 16.3° C. This area is beyond the immediate effect of the Fraser River and is within the sphere of influence of the cold saline waters from Discovery Passage at the north. At station 105 near the north end of Texada Island the salinity was lower, 13.36-13.80 gm. Cl, and the temperature was higher, 16.70 to 18.3° C., due to the effect of river water from northern inlets, especially Toba and Bute, which extend southward, particularly on the eastern side of the Strait.

The plankton quantity was low; at stations 101 and 103 readings of 7.5 units were obtained. The pH values were higher than might be expected, namely, 8.2 to 8.6. However, this may be accounted for by the proximity of phytoplankton either at lower levels or of laterally situated areas. North of the Fraser the salinity which is associated with optimal phytoplankton conditions varies from 11.0 gm. Cl, to 13.5 gm. Cl; that is, in the areas of

TABLE V

Salinity, temperature, pH and plankton, at 6 yards (5.5 metres) on a mid-channel line through Haro Strait, Strait of Georgia and Discovery Passage

Reference No.	Station	Location	Date	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
212	80	N. 48°32′, W. 123°12′	July 15, 1926	6:20 p.m.	F 6	12.35	17.10	8.15	—
213	76	N. 48°38′, W. 123°3′	July 15, 1926	10:54 a.m.	E 1	12.1	16.59	8.10	—
214	68		July 13, 1928	1:17 p.m.	F 5	13.18	14.13	8.20	12.5
215	68		Aug. 13, 1926	7:53 a.m.	F 6	11.72	16.52	8.20	6.0
216	68a		June 25, 1927	9:04 a.m.	F 1	9.96	16.73	8.05	2.50
217	68b		June 27, 1928	2:10 a.m.	F 3	16.40	13.74	8.53	60.0
218	68b		July 26, 1928	4:48 p.m.	E 2	15.17	14.47	8.35	45.0
219	68b		July 26, 1928	11:02 a.m.	F 3	14.38	14.15	8.25	15.0
220	54	N. 48°54′, W. 123°15′	July 17, 1929	7:32 p.m.	E 3	14.62	14.62	8.25	0.5
221	54		July 17, 1929	10:46 a.m.	F 2	12.90	15.34	8.10	0.2
222	54		July 30, 1926	4:04 p.m.	F 2	13.38	15.73	8.30	30.0
223	54		July 17, 1929	4:26 p.m.	E 1	10.75	16.51	7.75	0.5
224	55	N. 48°56′, W. 123°11′	Aug. 12, 1926	1:01 p.m.	E 6	15.37	14.52	8.48	—
225	53	N. 48°55′, W. 123°16′	July 16, 1926	2:07 p.m.	E 6	13.98	16.01	—	—
226	50	N. 48°58′, W. 123°23′	July 16, 1926	3:22 p.m.	F 1	13.9	15.81	8.28	—
227	45	N. 49°4′, W. 123°28′	July 11, 1928	5:35 p.m.	E 6	14.80	14.20	8.42	45.0
228	45		Aug. 6, 1926	4:49 p.m.	F 4	17.02	14.38	8.50	48.0
229	46	N. 49°5′, W. 123°24′	Aug. 6, 1926	5:58 p.m.	F 6	16.09	14.71	8.40	—
230	16		Aug. 10, 1926	2:10 p.m.	F 2	13.70	15.71	8.40	12.0
231	17	N. 49°15′, W. 123°33′	July 11, 1928	12:52 p.m.	F 6	16.17	11.24	8.35	5.0
232	17		July 8, 1927	1:34 p.m.	F 6	14.08	14.48	8.40	5.0
233	17		July 26, 1929	2:32 p.m.	F 1	13.20	15.40	8.15	—
234	8	N. 49°17′ W. 123°54′	Aug. 4, 1926	7:15 p.m.	E 3	16.53	14.82	—	—

235	1	N. 49°19′, W. 123°52′	Aug. 1, 1928	11:51 a.m.	F 1	16.60	14.39	8.60	—
236	1	10 mi. N.W. Departure	Aug. 18, 1927	8:37 a.m.	F 5	16.54	15.00	8.65	20.0
237	1	Bay	Aug. 4, 1926	5:38 p.m.	E 1	13.82	15.18	8.20	25.0
238	1		June 16, 1927	2:25 p.m.	—	11.35	15.69	8.20	—
239	1		Mar. 31, 1927	—	—	7.6	16.31	—	10.0
240	1		Dec. 17, 1926	—	—	7.7	16.68	8.0	—
241	10	N. 49°26′, W. 123°48′	Aug. 4, 1926	3:53 p.m.	F 6	13.60	15.29	8.25	64.0
242	2a		July 30, 1926	2:09 p.m.	F 5	16.42	14.42	8.35	0.5
243	2	N. 49°20′, W. 124°9′	July 23, 1926	6:27 a.m.	E 6	16.4	15.31	8.2	—
244	3	N. 49°24′, W. 124°12′	July 30, 1926	4:40 p.m.	E 1	15.55	15.10	—	2.0
245	3a		Aug. 3, 1926	7:10 p.m.	E 4	16.35	15.25	—	—
246	4	N. 49°28′, W. 124°10′	Aug. 4, 1926	7:51 a.m.	E 5	16.57	14.67	—	40.0
247	101	N. 49°28′, W. 124°29′	June 28, 1927	5:36 p.m.	F 6	15.17	13.60	8.48	25.0
248	103	N. 49°35′, W. 124°32′	June 28, 1927	3:53 p.m.	F 4	13.37	15.62	8.23	2.0
249	105	N. 49°41′, W. 124°43′	Aug. 6, 1928	4:36 p.m.	F 2	17.96	13.99	8.60	2.5
250	105	N.W. Texada Island	June 28, 1927	1:12 p.m.	F 3	14.66	14.12	8.32	20.0
251	107	N. 49°48′, W. 124°42′	June 28, 1927	11:37 p.m.	F 1	14.55	15.27	8.30	7.5
252	162	N. 49°39′, W. 124°48′	Aug. 10, 1928	12:34 p.m.	F 5	17.71	13.62	8.30	2.5
253	162	E. Comox Spit	Aug. 9, 1929	11:36 a.m.	E 4	15.81	15.58	8.30	0.5
254	110	N. 49°58′, W. 124°50′	Aug. 7, 1928	2:00 a.m.	E 5	17.8	13.23	8.40	6.2
255	110	N. Savary Island	Aug. 6, 1928	11:05 p.m.	E 1	17.7	13.52	8.50	5.0
256	110		Aug. 6, 1928	7:16 p.m.	F 5	17.59	13.66	8.55	6.0
257	110 n.e.		Aug. 12, 1929	9:33 a.m.	F 3	14.36	15.78	8.35	—
258	154	N. 49°54′, W. 125°5′	Aug. 9, 1929	2:30 p.m.	F 6	12.03	16.37	7.94	4.0
259	114	N. 49°59′, W. 125°10′	Aug. 9, 1929	5:31 p.m.	F 2	13.99	16.13	8.30	5.0
260	120	N. 50°1′, W. 125°14′ Quathiaski Cove	Aug. 8, 1928	4:50 a.m.	F 1	14.2	15.22	—	9.0
261	120	Discovery Pass	Aug. 7, 1928	11:38 a.m.	E 2	12.17	16.00	8.21	7.5
262	130	N. 50°10′, W. 125°22′	Aug. 8, 1928	11:12 a.m.	E 1	11.69	16.15	8.10	6.0
263	130	N. Seymour Narrows	Aug. 8, 1928	7:32 p.m.	F 2	11.42	16.30	8.10	5.0
264	133	N. 50°17′, W. 125°27′	Aug. 9, 1929	11:23 a.m.	E 2	10.20	16.85	7.82	1.0

primary mixing of river and sea water. The greater part of the area west of Texada Island has a higher salinity, the mixing has been completed and there is little phytoplankton. It is notable, however, that even small streams flowing into this region are accompanied by a very abundant plankton. The rivers entering at Courtenay, protected as they are by Comox Spit and Denman Island, give conditions which support plankton measuring 50+ units and the rivers entering at Qualicum and Parksville have similar effects, although less extensive, partly because of the unprotected shore line.

Northern Part of the Strait of Georgia

Water from two sources enters the northern part of the Strait, one through Discovery Passage and the other through Bute and Toba Inlets. The former source has high salinity and low temperature and enters at the western limit, while the latter has low chlorinity and high temperature during the summer period and enters the eastern area. Between is a region of turbulence which is increased by the meeting of the tidal flood currents, the one from the south and the other from the north. The tide from the south continues through Sutil Channel and into Bute and Toba Inlets, that is, for more than 60 miles. The tidal current through Discovery Passage may attain 12 knots. In part at least the mass of water which accumulates at the northwestern region of the Strait of Georgia may account for the well-known general eastward drifts in this region.

Certain eddy regions occur where there is practically no tidal change. At station 110, Savary Island, Aug. 6-7, 1928, a twelve-hour series of samples was collected with a constant temperature of 17.8° C. except for one reading, 17.5° C., and the variation to a depth of 10 yards was not more than 0.4° C. Similarly the chlorinity to the same depth was constantly within the limits 13.26 to 13.65. The plankton at the surface remained between 5.0 and 6.0 units, and the pH at 8.35 to 8.40. The conditions at this station were the most nearly constant observed in the Strait of Georgia.

Conditions on the west side of the Strait are illustrated by records at station 162, off Cape Lazo, 25 miles from the entrance to Discovery Passage, which had a temperature 18.4° C. and chlorinity 13.04 gm. Cl on the flood tide and shows the effect of the Fraser River while on the ebb the characters of the water from Discovery Passage become apparent with temperature 16.74° C. and chlorinity 15.55 gm. Cl. The line of separation between waters from the south and from the north evidently shifts on either side of this station from one phase of the tide to another. At station 115 the low chlorinity and high temperature, 14.56 gm. Cl and 17.8° C., occurred on the ebb and the reverse on the flood, 16.00 gm. Cl and 15.20° C. The water of low salinity comes from Sutil Channel and that of high from Discovery Passage. Tides of the same phase have reverse effects on opposite sides of the separating line according to the source of the flood waters, from the south or from the north.

A series taken at the beginning of the flood, Aug. 9, 1928, from the mouth of Sutil Channel, station 149, in a direction southwesterly to station 153, a dis-

tance of eight miles, showed a temperature change from 19.9 to 16.9° C. and a chlorinity change from 12.83 to 15.0 gm. Cl. A westerly series would show a similar change within a much less distance.

Discovery Passage

The temperature and chlorinity are relatively uniform. At Quathiaski Cove, which is nearly opposite Campbell River, the chlorinity varied only from 15.17 to 16.71 gm. Cl and the temperature from 14.40 to 10.54° C. at the surface during a 24-hr. series. The river water which enters is so distributed by the turbulence that its effect is dissipated. An eight-hour series showed a change of chlorinity from 15.77 to 16.30 gm. Cl, accompanied by a pH change from 8.27 to 8.00 with constant or slightly increased phytoplankton.

At station 130 north of Seymour Narrows a 24-hr. series showed a temperature variation of 11.2 to 12.0° C. and chlorinity 16.04 to 16.34 gm. Cl, and at station 133, at the entrance to Okisallo Channel, a temperature 10.45 to 11.8° C. and chloride 16.16 to 16.79 gm. Cl were recorded. The plankton was sparse, 0.5 units on the ebb with a pH from 7.80 to 8.15. It may be noted that while the surface salinity is lower than that sometimes found in Haro Strait, (cf. 16.79 and 17.35 gm. Cl), the temperature is lower than in Haro Strait during the summer, (cf. 10.45 and 12.40° C., using the maximal readings for chlorinity and the minimal for temperature).

Salinity, Temperature, pH and Plankton at a Depth of 6 Yards (5.5 Metres) on a Line Through Haro Strait, Strait of Georgia and Discovery Passage

The readings at six yards (5.5 metres) give a fair estimate of the depth of the epithalassa. At the time of the flood waters of the Fraser River, June 27, 1928, chlorinity at station 68 was 13.74 at six yards depth, on the mid-flood, while August 13, 1926, it was 16.73 gm. Cl. This is in agreement with observations recorded by Thompson (15) who reports that at certain periods water of decidedly low salinity is found as far south as the entrance to San Juan Passage. This low salinity water of June 27, 1928 also had a high temperature, 16.40° C., and an exceptionally high plankton content of 65 units, and pH of 8.53. The evidence would indicate that the source was the region eastward, as explained before, and not southward as one might at first suppose. The early flood moves from the south as indicated by the sample taken at six yards on June 25, 1927, when the chlorinity was 16.73 gm. Cl, the temperature was 9.96° C., the plankton 2.5 units and the pH 8.05. Station 54 shows something of the same effect of the swirl off the Boundary Bay. On July 17, 1929, the beginning of the ebb gave a high chlorinity, 16.51 gm. Cl; low temperature, 10.75° C.; low pH 7.75; and plankton, 0.5 units, having apparent characteristics of sea water.

The regions which show the most marked characteristics at depth of six yards are found westward and northward of the Fraser River mouth. At station 45, Aug. 6, 1926, the temperature was 17.02° C. and chlorinity 14.38 gm. Cl, and at station 17, July 11, 1928, was 16.17° C. and 11.24 gm. Cl.

TABLE VI

Surface temperature, salinity, pH and plankton on a line from Snake Island, Nanaimo, to Vancouver

Reference No.	Station	Location	Date	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
265	13	N. 49°13′, W. 123°52′	July 11, 1928	9:42 a.m.	F 2	18.3	9.98	8.5	20.0
266	13b	S.E. Snake Island.,	July 11, 1928	10:10 a.m.	F 3	18.2	11.17	8.50	—
267	13c		July 11, 1928	10:21 a.m.	F 3	19.2	10.30	8.50	—
268	14	N. 49°13′, W. 123°45′	July 11, 1928	10:05 a.m.	F 3	19.5	10.23	8.50	30.0
269	14a		July 11, 1928	11:31 a.m.	F 3	19.4	9.90	8.42	25.0
270	15	N. 49°14′, W. 123°41′	July 11, 1928	11:42 a.m.	F 4	19.5	10.24	8.42	20.0
271	15a		July 11, 1928	11:53 a.m.	F 5	19.7	8.98	8.45	—
272	16	N 49°15′, W. 123°37′	July 11, 1928	12:03 p.m.	F 5	19.6	8.91	8.42	12.5
273	16a		July 11, 1928	12:14 p.m.	F 5	20.1	8.90	8.52	—
274	17	N. 49°15′, W. 123°34′	July 11, 1928	1:02 p.m.	F 6	20.5	8.34	8.33	10.0
275	17a		July 11, 1928	1:12 p.m.	F 6	20.45	7.26	8.40	—
276	18	N. 49°16′, W. 123°30′	July 11, 1928	1:23 p.m.	F 6	20.80	7.05	8.30	2.5
277	19	N. 49°16′, W. 123°25′	July 11, 1928	1:45 p.m.	E 1	21.75	8.18	8.35	5.0
278	19a		July 11, 1928	1:56 p.m.	E 1	21.4	7.48	8.38	—
279	20	N. 49°17′, W. 123°21′	July 11, 1928	2:46 p.m.	E 2	22.10	7.50	8.40	7.5
280	20b		July 11, 1928	2:46 p.m.	E 2	23.20	6.13	8.32	—
281	20c		July 11, 1928	2:56 p.m.	E 2	23.70	4.63	8.27	0.2
282	40	N. 49°12′, W. 123°20′	July 11, 1928	3:15 p.m.	E 2	22.5	3.15	8.20	0.2
283	40		July 11, 1928	3:16 p.m.	E 2	22.0	1.77	8.20	—
284	20	N. 49°17′, W. 123°21′	July 6, 1928	3:05 p.m.	F 1	20.5	4.60	—	1.0
285	21	N. 49°17′, W. 123°18′	July 6, 1928	2:40 p.m.	F 1	18.0	2.51	—	1.0
286	23	N. 49°18′, W. 123°8′	July 6, 1928	2:14 p.m.	F 1	16.95	4.32	8.25	1.0
287	24	N. 49°18′, W. 123°5′	July 6, 1928	11:48 a.m.	E 5	15.24	9.05	8.25	2.0
288	24		Aug. 11, 1926	7:50 a.m.	E 1	15.3	13.38	8.25	10.0

The chlorinity mentioned for station 17, which is 20 miles north of the river mouth, is the lowest recorded for this depth. In this area the upper six yards are fairly uniform while between the depths of six and ten yards there is a rapid increase in salinity and decrease in temperature.

Northward there is a very narrow normal range of chlorinity, 14.0 to 15.6 gm. Cl, while the temperature varies from 13.2 to 16.6 ° C. At the north and south ends of Texada Island however, stations 101 and 105, the chlorinity is low, 14.0 and 13.6 gm. Cl. It would appear that some distance from the sources of fresh water, namely, Bute and Jervis Inlets, the six yard level attains a low salinity as in the case near the Fraser cited above. At Savary Island, station 101, and off Cape Lazo the effect of the river water emerging from the inlets at the north is even more marked. The temperatures were 17.71 and 17.8° C. respectively, while the chlorinities were 13.62 and 13.23 gm. Cl.

Temperature, Salinity, pH and Plankton on a Line from Snake Island, Nanaimo, to Vancouver (Table VI)

This series of surface samples was collected July 11, 1928. There may be noted a *gradual increase of temperature* from 18.2° C. at Snake Island to Station 20, located two miles west of the entrance of Burrard Inlet, where the temperature was 23,70° C., while at station 40, opposite the north arm of the Fraser River, a somewhat lower temperature of 22.0° C. was observed. On July 6, 1928, a series from the entrance of Burrard Inlet, station 20, to Vancouver Harbor showed a sudden decrease in temperature at the Narrows, that is, from 18.0 to 15.24° C. This series shows that the maximum temperature is attained about 20 miles from the Fraser River after insolation has had time to intensify its effect and beyond there is a gradual decrease, which may be shown by the salinity to result from mixing with colder sea water. The temperatures also indicate that very little surface river water goes through the passes. As explained in a previous paper by Lucas (12) and Hutchinson (9) there is a "piling up" at the narrows and a "sliding back" of the upper, less saline layer.

In general the chlorinity decreased from 11.17 to 1.77 gm. Cl from Snake Island across the Strait to the north arm of the Fraser River. The most notable feature, however, was the occurrence of successive crests of higher salinity at distances of ten to twelve miles; in each case there was a rather abrupt increase to the high salinity followed by a gradual decrease eastward, for instance, 8.98, 11.17, 10.30, 10.23, 9.90 gm. Cl for the first wave; followed by 9.90, 10.24, 8.98, 8.91, 8.90, 8.34, 7.26, 7.05 gm. Cl for the second wave; and 7.05, 8.18, 7.48, 7.50, 6.13, 4.63, 3.15, 1.17 gm. Cl for the third wave. On a calm day corresponding regions of successively less turbidity may be seen as one proceeds from the river across the Strait. These have abrupt western limits and are chiefly the result of the water carried north and westward by successive tides. These tidal "waves" are longer northwestward than westward from the river.

The surface plankton showed a single crest on this line at station 14, 25 miles from the river mouth, with 30 units of phytoplankton. There was a gradual decrease toward the river with a scarcely measurable value of 0.2 at station 40, and there was a correspondingly gradual change in pH from 8.50 to 8.20, as the salinity and the amount of plankton decrease.

TABLE VII

SURFACE SERIES, STRAIT OF GEORGIA TO HEAD OF HOWE SOUND (Aug. 9, 1927)

Station	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
21	10:20 a.m.	F 3	17.40	4.49	8.22	1.2
31	11:10 a.m.	F 3	17.0	4.34	8.41	3.0
30	2:20 p.m.	F 6	16.00	2.64	7.85	0.5
29	4:22 p.m.	E 2	9.50	0.03	7.55	0.2
30	6:14 p.m.	E 5	15.60	2.09	—	0.5
31	7:12 p.m.	E 6	17.80	4.55	8.45	—

Surface Series

Strait of Georgia to the Head of Howe Sound (Table VII)

The temperature decreases gradually along a line from the mouth of Howe Sound to station 30, ten miles or less from the head, that is, from 17.40 to 16° C. and then decreases rapidly to the mouth of the Squamish River which is fed by glaciers; the temperature on August 9, 1927 was 9.50° C. The first series was taken on the flood tide and the second on the ebb of the same day with approximately the same values for the same station, which would indicate that there is relatively little tidal effect in a sound with a wide mouth, as in this case; other data support this opinion.

The salinity varied from 4.49 gm. Cl at the mouth of the Sound to 0.03 gm. Cl at the head; these values are lower than others obtained at other times in the

TABLE VIII

SURFACE SERIES, DEPARTURE BAY TO BOUNDARY PASS VIA TRINCOMALI CHANNEL (AUG. 16, 1929)

Station	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
35	8:55 a.m.	F 1	17.40	15.10	8.65	10.0
35g	9:15 a.m.	F 1	17.00	15.20	8.65	9.5
43n	9:45 a.m.	F 1	16.90	15.15	8.30	5.5
43s	10:20 a.m.	F 2	15.00	15.40	8.00	1.5
49	10:05 a.m.	F 2	16.90	15.08	8.60	13.5
52g	11:45 a.m.	F 3	16.41	15.28	8.42	19.0
52k	12:30 p.m.	F 3	14.80	15.81	8.22	1.5
68c	1:05 p.m.	F 3	12.20	16.98	7.90	2.0
68d	1:40 p.m.	F 4	12.20	16.95	8.00	—

same area. The plankton was very low in quantity at the surface. The pH showed the direct effect of the presence or absence of sea salts, pH 7.55 for chlorinity 4.49 gm. Cl.

Further study of this area is being conducted.

Stuart Channel to Boundary Pass, via Trincomali Channel and Plumper Sound (Table VIII)

During this series two marked crests in temperature, plankton and pH may be noted. In Stuart Channel the values are high; temperature, 17.40° C.; pH, 8.65; and plankton, 10 units, with a correspondingly low chlorinity, 15.10 gm. Cl. There is a gradual change to station 43s, a mile south of Porlier Pass, where the values are: 15.0° C.; pH, 8.00; plankton, 1.5 units; and chlorinity, 15.40 gm. Cl. Then an abrupt change to station 49, opposite the mid-point of Galiano Island with readings; 17.90° C.; pH, 8.60; plankton, 13.5 units; and chlorinity, 15.08 gm. Cl; and finally a gradual change through Plumper Sound to Boundary Bay with records of 12.20° C.; pH, 8.00; 2.0 or less units of plankton and 16.98 gm. Cl. The relation of these characteristics is most evident; temperature, plankton and pH decrease with an increase of chlorinity in this area while a decrease in chlorinity is accompanied by increase of the other characteristics. It may be noted that these readings were taken beginning with the first of the flood tide and continuing past mid-tide. The regions of low chlorinity are north of Porlier and Active Passes respectively, and the river water which entered during the preceding ebb is carried northward by the flood; the areas of high chlorinity are composed essentially of water, such as comprises the great mass within these channels, originating from Haro and Juan de Fuca Straits.

TABLE IX

SURFACE SERIES, SOUTH OF BOUNDARY PASS TO STRAIT OF GEORGIA (AUG. 17, 1929)

Station	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
68s3	11:45 a.m.	F 1	12.70	16.59	7.82	1.5
68s2	12:20 p.m.	F 1	13.10	16.30	7.90	1.0
68s2	12:25 p.m.	F 1	12.70	16.49	7.90	1.0
68	12:30 p.m.	F 2	12.90	16.44	7.90	2.0
68n1	12:45 p.m.	F 2	15.20	15.32	8.02	—
68n2	12:50 p.m.	F 2	17.20	13.73	8.25	—
68n3	1:02 p.m.	F 3	18.90	12.05	8.30	1.5
68n4	1:18 p.m.	F 3	18.80	11.40	8.40	1.0
68n5	1:35 p.m.	F 3	19.30	12.18	8.40	0.5

South of Boundary Pass to the Strait of Georgia (Table IX)

This series has been mentioned previously but a number of additional facts may be deduced. The boat, "A. P. Knight" travels about eight knots, consequently the total distance represented in the table is about sixteen miles and within six miles the change took place from typically Juan de Fuca or

sea water to Strait of Georgia water, showing the effect of the Fraser River. The most notable change occurred in less than two miles off East Point, namely: temperature, 12.90 to 17.20° C.; chlorinity, 16.44 to 13.73 gm. Cl; and pH, 7.90 to 8.25.

TABLE X

SURFACE SERIES, JOHNSTON STRAIT TO BUTE INLET (AUG. 10, 1929)

Station	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
133	11:41 a.m.	E 2	10.45	16.79	7.80	0.5
134	12:40 p.m.	E 3	10.80	16.71	7.82	0.5
135	1:52 p.m.	E 4	10.57	16.57	7.79	1.0
136	2:30 p.m.	E 6	13.70	11.43	8.08	3.0
137	2:55 p.m.	F 1	16.90	5.18	8.50	1.0
138	5:10 p.m.	F 3	14.00	2.90	8.05	0.5
140	7:41 p.m.	F 5	16.22	2.31	7.70	0.2

TABLE XI

SURFACE SERIES, JOHNSTON STRAIT TO BUTE INLET (AUG. 9, 1928)

Station	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
133	9:40 a.m.	F 5	11.8	16.16	8.13	2.5
134	10:00 a.m.	F 5	11.6	15.95	8.08	2.5
135	10:30 a.m.	F 5	11.2	16.16	8.08	5.0
137	12:25 p.m.	F 6	14.0	5.35	8.45	5.0

Johnston Strait to Bute Inlet (Tables X and XI)

On August 9, 1928, and August 10, 1929, surface series from Johnston Strait, station 133, through Okisollo Channel, and through the Hole-in-the-Wall to Bute Inlet were collected, the former on the flood tide and the latter on the ebb. The most marked feature is the sudden change in chlorinity and temperature as one passes through the Hole-in-the-Wall, a distance of four miles; *e.g.*, chlorinity from 16.57 to 5.18 gm. Cl and temperature from 10.57 to 16.90° C. At Orford Bay, 20 miles up the inlet, the temperature was 16.22° C. and the chlorinity 2.31 gm. Cl. Although glacial water enters the inlet, especially at the head, the stability of the epithalassa makes possible a high surface temperature due to insolation.

The temperature was higher by more than 1° C. and the salinity lower by 0.6 gm. Cl on the flood as compared with the ebb in Okisollo Channel. It would appear that less saline water is carried from Johnston Strait, probably from the Salmon and other neighboring rivers; or it may be that the water column observed on the ebb had become more uniform due to turbulence in the narrow passes.

There is little surface plankton, 0.5 to 1.5 units, the chlorinities being extremely high. The pH shows a maximum with intermediate salinity as previously described.

Bute Inlet to Sutil Channel and the Strait of Georgia to Comox Harbor (Tables XII and XIII)

This series was collected during the ebb, August 11, 1929. The effect of the tide is illustrated by comparison of the surface data at station 137, at the mouth of Bute Inlet, with those at the beginning of the flood on the preceding day (cf. Table X). On the early flood the temperature was 16.90° C. and the chlorinity, 5.18 gm. Cl, while on the early ebb the values were 12.42° C. and 11.97 gm. Cl. Since the readings were taken shortly after the turn of the tide, they represent the effect of the previous phase to a great extent. There is a gradual increase in salinity from the mouth of Bute Inlet through Sutil Channel to the Strait of Georgia, namely, from 11.97 to 15.60 gm. Cl. The effect of the saline water entering through Discovery Passage is evident. There is a rapid decrease in salinity from the mouth of Sutil Channel eastward to Savary Island, that is, from station 147 to 110; the change is 15.60 to 13.78 gm. Cl.

The gradual increase in salinity is accompanied by a rapid increase in temperature from 12.42 to 21.20° C.; that is, the heating effect of insolation more than counterbalances the cooling effect of ocean water which enters through Discovery Passage with a temperature approximately 12.5° C. On the other hand there is a lowered temperature opposite Calm Channel, station 142, which may be due to colder waters entering from the not distant glacial sources of Bute Inlet, or may be caused chiefly by the turbulence due to the meeting of currents, bringing colder water from a lower depth. The temperature at this point for two successive years, 1928 and 1929, Tables XI and XII, was more than 2° C. lower than at points two miles either north or south.

Although there was very little surface phytoplankton the depth samples which were taken indicate that the increasing pH, from 8.25 to 8.62, was the result of a corresponding increase of diatoms and peridinians immediately below the surface.

Sutil Channel to Comox Harbor (Table XIII)

The series began at the entrance of Calm Channel then to Sutil Channel, continued through Sutil Channel south across the northern part of the Strait of Georgia to Cape Lazo and finally inside Comox Spit. From station 142 to 147 the series duplicated that of Table XII except that it was taken one day later in the same month of the preceding year and at an earlier part of the ebb tide. In Sutil Channel the salinity is lower and the temperature higher showing the effect of the river water which has been warmed through insolation. The temperatures were 14.0 to 20.4° C. and the chlorinities, 8.77 to 12.98 gm. Cl. The higher salinity at station 145 is probably due to sea water which has entered through Okisollo Channel. A similar and more marked increase in salinity, in this case accompanied by a decrease in temperature, is evident at station 157 as a result of the water which flows through Discovery Passage; the changes are from 20.2 to 16.8° C. and from 12.98 to 15.30 gm. Cl.

While crossing Comox Spit the chlorinity is decreased from 15.30 to 15.03 gm. per litre; the temperature increased from 16.8 to 18.2° C. Although the waters from the Comox and Tsolum Rivers lower the chlorinity of the waters protected by Denman Island less than 0.3 gm. Cl per litre, this change is accompanied by a remarkable increase in plankton from 4.0 units to 25.0 units with a corresponding increase in pH. The temperature near the entrance of the River is 16.6° C., or 1.6° C. less than in the bay where sufficient time has made possible the heating effect of insolation on the surface water.

Surface Isohalines for the Strait of Georgia and Neighboring Waters during July and August at Flood Tide

Chart 2 is an attempt to put into graphical form the average surface chlorinity conditions during the months of July and August. It is recognized that it is difficult to represent a dynamic situation dependent upon many variable factors in a static chart. Moreover although many samples have been collected over this extensive area the diagram attempts to summarize only the data collected to date. In fact, a number of areas are being investigated in greater detail at present. The survey is admittedly general.

TABLE XII

SURFACE SERIES, BUTE INLET TO STRAIT OF GEORGIA (AUG. 11, 1929)

Station	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
137	11:51 a.m.	E 1	12.42	11.97	8.25	—
137s1	12:15 p.m.	E 1	13.70	12.48	8.24	—
137s2	12:30 p.m.	E 2	16.30	11.39	8.30	0.3
142	2:01 p.m.	E 5	14.42	13.14	8.42	—
143	3:30 p.m.	E 6	16.80	13.48	8.50	—
147	4:30 p.m.	E 6	18.80	15.60	8.62	—
110	4:25 p.m.	F 1	21.20	13.78	8.60	—

TABLE XIII

SURFACE SERIES, SUTIL CHANNEL TO COMOX HARBOR

Station	Date	Time	Tide	Temp., °C.	Chlorine, grams per litre	pH	Plankton, volumes per 100,000
142	Aug. 9, 1928	3:05 p.m.	E 2	14.0	8.77	—	2.5
143	Aug. 9, 1928	3:35 p.m.	E 3	18.0	9.95	—	5.0
144	Aug. 9, 1928	3:50 p.m.	E 3	19.0	9.88	—	5.0
145	Aug. 9, 1928	4:20 p.m.	E 3	20.4	13.40	—	2.5
147	Aug. 9, 1928	4:50 p.m.	E 5	20.2	12.98	—	2.5
157	Aug. 9, 1928	7:50 p.m.	F 3	16.8	15.30	—	4.0
160	Aug. 9, 1928	9:23 p.m.	F 5	18.2	15.03	8.45	25.0
159	Aug. 10, 1928	10:38 a.m.	F 3	16.6	15.0	8.28	21.0

(a) Salinities of the Area Dominated by the Fraser River

The greater part of the southern portion of the Strait of Georgia is dominated as to salinity by the Fraser River during the summer months when the mass of fresh water contributed is the greatest and when the low specific gravity of the warmer, less saline water provides conditions for a stable epithalassa. The

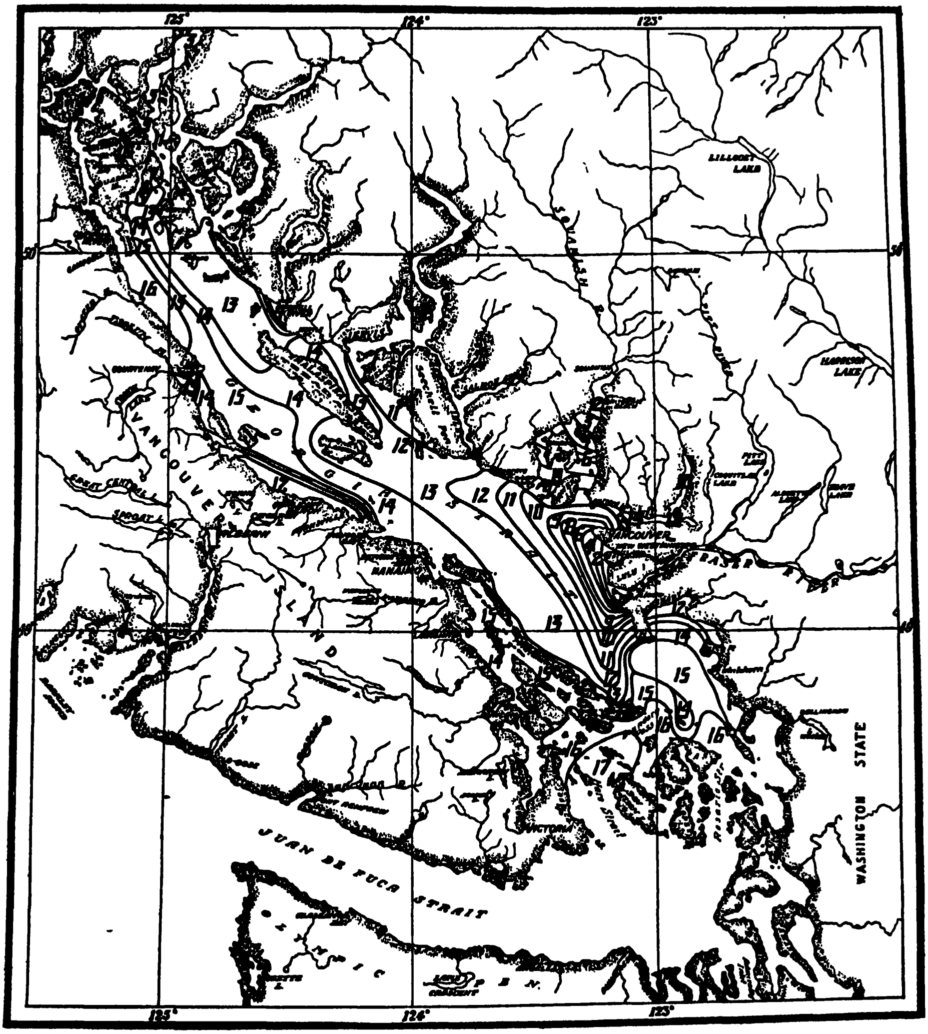


CHART 2. *Salinity, Strait of Georgia.*

surface water as it enters has a chlorinity less than 1.0 gm. About this centre the isohalines form an irregular fan-shaped figure, the radii being least westward and southeastward, greater southwestward and greatest northwestward; the distances are approximately 10, 20 and 40 miles for chlorinity 12.0 gm. Cl. The northwest and southeast tidal movements explain these phenomena.

The region with a chlorinity of 13.0 gm. occupies nearly one-half the total region extending from a narrow tip at the east of Saturna Island to Lasqueti and Texada Islands as an ever widening area. The region with chlorinity 14.0 gm. Cl forms what may be regarded as the transition region from that dominated by the Fraser River to those controlled by other factors; it extends first as a narrow fringe along Saturna, Mayne, Galiano, Valdes and Gabriola Islands, and as a wider area northward to the central point of Texada Island. In June, these isohalide areas extend farther because of the high water of the Fraser (12).

(b) Salinities of the Areas Dominated by Rivers Fed by Glacial Streams

The most notable of those studied are Bute Inlet and Howe Sound.

Bute Inlet. At Orford Bay which is only half the distance up Bute Inlet the chlorinities are as low as 3.0 gm. Cl. This was the most distant observation. There is a gradual increase through Sutil Channel and at the mouth the chlorinity is 13.0 gm. Cl. A similar condition obtains southward on the east side of the Strait of Georgia to the north end of Texada Island a distance of 40 miles; the total distance from the head of the inlet is more than 100 miles (160 km.). Toba Inlet is probably similar and contributes to the general effect within the Strait of Georgia, but has not been investigated.

Howe Sound. The Squamish River with its tributary the Cheakamus River lowers the chlorinity at the head of Howe Sound to less than 2.0 gm. per litre and its influence is shown, especially on the ebb tide, to the mouth of the sound where its effect merges with that of the Fraser River. The total distance is 25 or 30 miles. Although the water discharge of the Squamish River is probaby greater than that of the rivers flowing into Bute Inlet, the influence is less evident partly because the Sound has less length and has a wide mouth and partly because of the proximity of a greater factor, the Fraser River. Further investigation of this area is being conducted.

Indian Arm. The chlorinity of Indian Arm, Burrard Inlet, is lowered by the Indian River to the extent of 4.0 gm. Cl per litre at four miles from the head although the river is small. Vancouver Harbor has a much higher chlorinity than English Bay or the waters of Indian Arm. The primary effect of the Indian River does not pass the Second Narrows and that of the Fraser is held back by the First Narrows, or Lion's Gate, due to the "piling up" of sea water and the "sliding back" of surface river water as described above. The high chlorinity (14+gm. Cl) along the north shores of Stanley Park is such that the kelps, as *Nereocystis*, and animals such as starfish, sea urchins and tube worms thrive, while in English Bay and neighboring waters there is a paucity of marine forms. The small streams Capilano, Seymour and Lynn which enter at the narrows become thoroughly mixed with the sea water and do not form a definite epithalassa.

Jervis Inlet. This inlet receives a number of streams whose effect is such that at the mouth the chlorinity is 11.00 gm. Cl and at the junction of the area of its influence and that of the Fraser River, that is, toward the southern portion of Texada Island, the combined effect gives a chlorinity 12.0 gm. per litre.

Powell River. This river empties into the open Strait. The chlorinity of 5.0 gm. per litre at its mouth changes within a distance of two miles seaward to the general chlorinity of 13.0 gm. Cl.

(*c*) *Salinities of the Areas Dominated by Rivers Fed by Upland Lakes*

The rivers of the east coast of Vancouver Island are relatively small and are fed by lakes, consequently the quantity of their water supply although small is more uniform. The effect of the Chemainus River is the most marked chiefly because it flows into a protected area, namely, Stuart Channel; consequently the river water is conserved. The Comox and Tsolum Rivers have a similar effect on Baynes Sound, between Denman Island and Vancouver Island. In each case the chlorinity of these considerable bodies of water is lowered at the surface by 1.0 to 2.0 gm. Cl per litre.

The Horne, Qualicum and Englishman Rivers in the Qualicum Beach area affect only the area near the shore because of the more open nature of the shore line. However, Denman and Hornby Islands offer some protection from tidal currents.

Campbell River enters Discovery Passage near Seymour Narrows and although it has considerable volume its effect cannot be detected easily at Quathiaski Cove on the opposite shore. The turbulence due to tidal currents causes immediate mixing.

Similarly the Cowichan River has little effect beyond the limits of Cowichan Bay. A special study of Saanich Arm and Cowichan Bay is being made by Carter.

The influence of the Serpentine and Nicomekl Rivers is marked over the tide flat area only. Because of the importance of this region for oyster beds a separate investigation is being conducted by Elsey.

(*d*) *The Salinities of Areas Dominated by Sea Water*

The sea water which enters through Juan de Fuca Strait has a chlorinity 17.0 or more gm. Cl per litre; it enters by Rosario Strait and Boundary Pass into the Strait of Georgia and is evident until at the Point Roberts-Saturna Island line it has a halide value of 14.0 gm. Cl. This may be regarded as the line of transition between the dominance of the sea and of the river. The effect of the current through Rosario Strait extends farther since the current continues one hour longer. An area of lower chlorinity is situated near Sucia Island and is the result of the tidal swirl of the Boundary Bay region which apparently surrounds small areas of river water and at the flood of the river may carry them southward through Boundary Pass. Ordinarily however the river water is conserved in the Strait because of the "piling up" effect at the passes on the ebb tide.

The water from Haro Strait also enters Swanson Channel (chlorinity, 16.0 gm. Cl), and continues through Trincomali Channel with chlorinity 15.0 gm. Cl and another branch flows into Saanich Inlet. The narrow neck of water dominated by the sea, extending through Trincomali Channel more than 30 miles and separated from Georgia Strait by islands interrupted by

narrow passes, has a chlorinity 2.0 gm. Cl per litre greater than adjacent waters. This gives an excellent example of the closure to fresh water effected by constricted passes.

The sea water from Johnston Strait which enters through Discovery Passage has a lower salinity (16+gm. Cl) than that from the south through Juan de Fuca. Its effect on the waters of the Strait of Georgia is limited chiefly to the western portion as far south as Cape Lazo (chlorinity, 16 gm. Cl); while the area of 15-gm. chlorinity extends beyond Hornby Island. There is an eastward drift, especially at the north, where this area of influence comes in contact with that dominated by the rivers of Bute and Toba Inlets.

Surface Isotherms, Strait of Georgia

Chart 3

The chart represents the average surface temperature near the final phase of the flood tide during the months of July and August.

Area Dominated by the Fraser River

This area is situated with its centre 20 miles north of the river's mouth and has the form of a triangle. The northern angle extends nearly to Texada Island, the southern to Saturna Island and the eastern to the entrance of Burrard Inlet; more than four-fifths of the area is north of the main channel of the river. The surface river water meets a barrier at the southern passes and floats northward, propelled by the current of the flood tide on the east side of the strait, and southward on the west side carried by the final phases of the flood and by the ebb. The river water has an average summer temperature slightly greater than 16° C. as it enters the strait; it becomes warmed by insolation to 22° C. or higher as it approaches the centre of its distribution, and gradually decreases to 18° C. as it becomes mixed with the waters from the north; this transition area extends along the west shores of Mayne, Galiano, Valdes, and Gabriola Islands, and northward to surround Texada Island.

Areas Dominated by Rivers of Glacial Origin

These have much lower temperatures near the mouths. At the head of Howe Sound the temperature is 10° C. and there is a rapid increase due to radiation until the waters of the Squamish combine with those of the Fraser to make possible the high temperature, 22° C. Indian Arm, Burrard Inlet, has a higher temperature near its head, 14° C.; since it receives a small stream only the epithalassa is not very stable and the maximum temperature is 16° C. The rivers flowing into Bute Inlet lower the temperature at its head very materially; at Orford Bay more than 20 miles from the head the temperature varies from 10 to 15° C. according to the tide; at the mouth of the inlet it is 17° C. and becomes 19° C. at the mouth of Sutil Channel. This temperature is maintained to the north end of Texada Island. In the eddy about Savary Island a temperature of 21° C. is attained.

Areas Dominated by Rivers Originating from Upland Lakes

These areas receive river water which has been warmed previously to some extent and which enters at a temperature approximately 17° C. Provided

this is conserved by barriers it soon becomes warmed to 20° C. Cowichan Bay, Stuart Channel, Qualicum Beach, Baynes Sound, and Boundary Bay are included in the group of areas so affected.

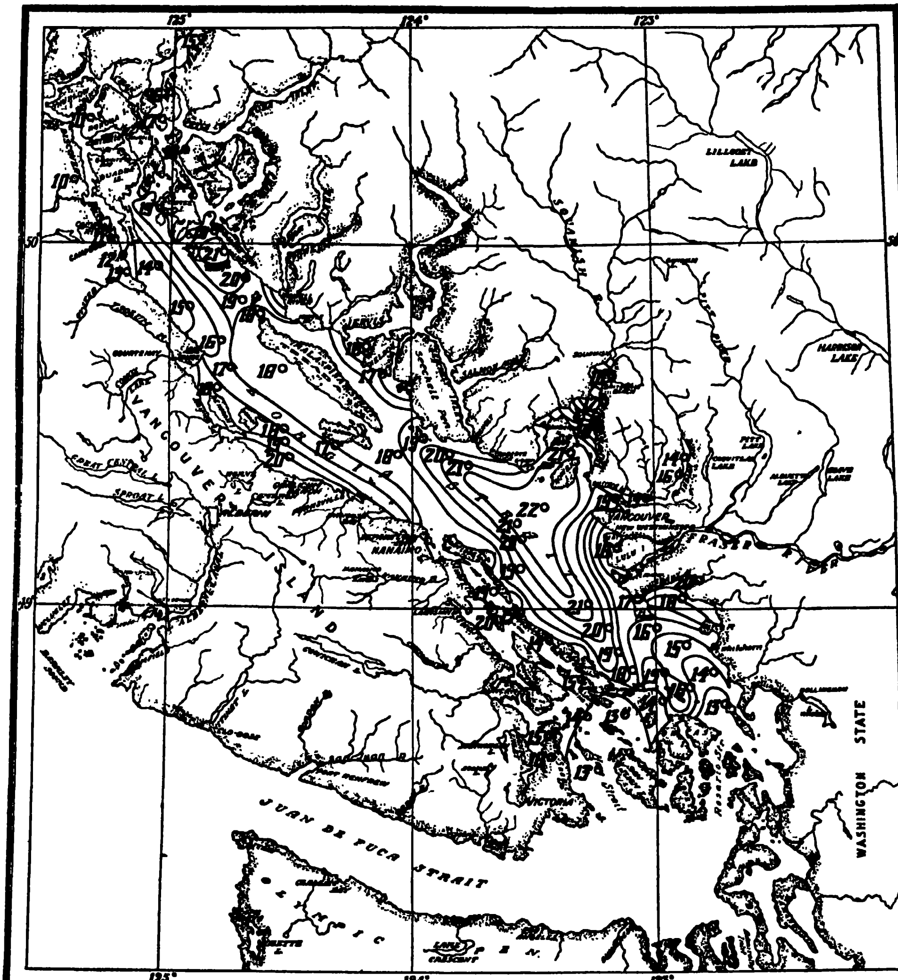


CHART 3. *Temperature, Strait of Georgia.*

Areas Dominated as to Temperature by the Sea Water

These are similar in outline to the salinity areas. The waters of Haro and Rosario Straits, with a temperature 13° C. extend their influence to the Point Roberts-Saturna Island line where the mixing with the Fraser water produces a temperature of 16° C. A long neck extends up Trincomali Channel which maintains a temperature of 15° C. even to Porlier Pass.

The water entering by Discovery Passage has a lower temperature than that entering at the south, namely, 10° C., and it affects the western side of the Strait of Georgia as far south as Cape Lazo, at 16° C., and to the Ballenacs Islands, at 17° Ç.

In general the temperature depends upon the time and degree of insolation as well as the temperature of the source. The time during which heating may continue depends upon the stability of the epithalassa. The temperature, although often related to chlorinity, is not necessarily an expression of the salinity. Starting with a source such as the Fraser River or Bute Inlet the temperature increases with increased chlorinity until its maximum is reached and from that point it decreases with increased chlorinity. Although the chlorinity of Saanich Arm (station 74) is high even near its head, the temperature reaches 18° C. or higher since it is subject to very minor currents only at its upper reaches. Ladysmith Harbor (station 42) is similarly affected.

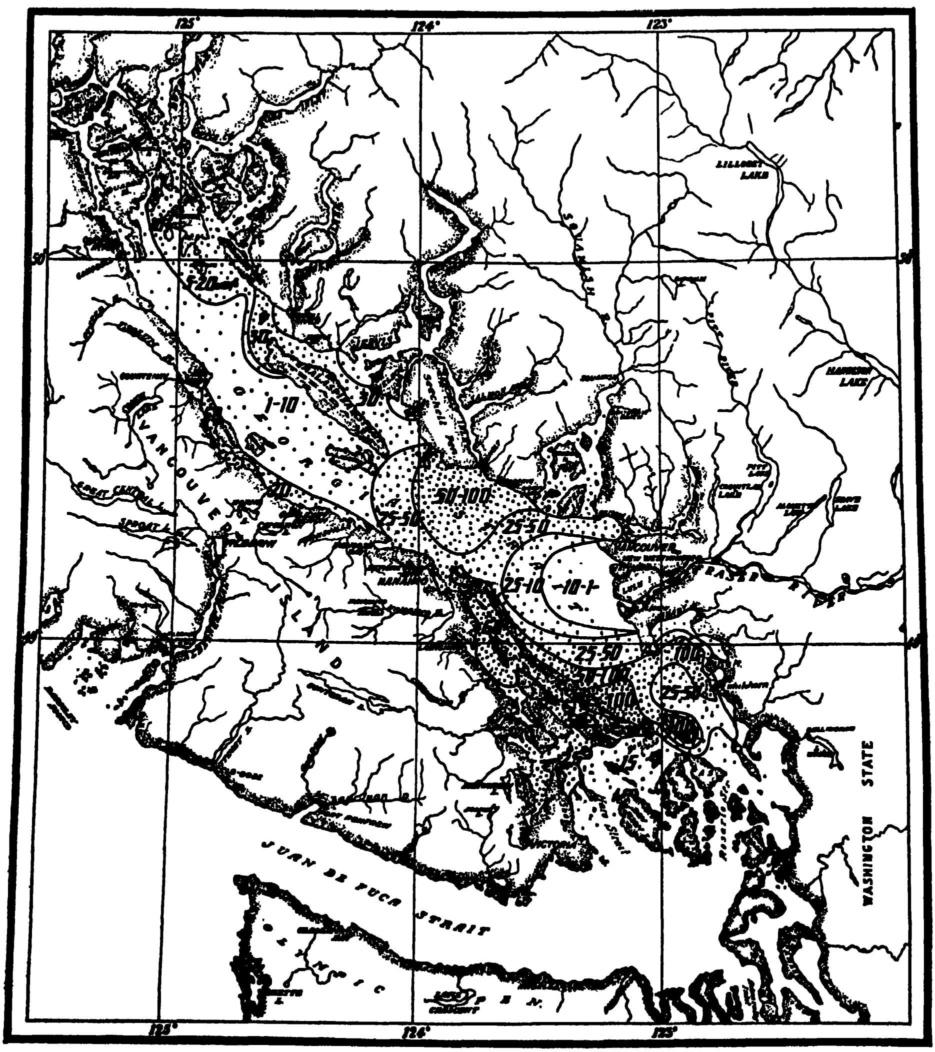


CHART 4. *Phytoplankton, Strait of Georgia.*

TABLE XIV
Chlorine, Strait of Georgia, S.W. (Fig. 1)

Station		133	130	120	114	154	105	103	3	1	14	44	54	68n	685	76	80
Date		9/7/29	8/7/28	7/7/28	9/7/29	9/7/29	6/7/28	28/5/27	3/7/26	4/7/26	10/7/26	30/6/27	30/6/26	13/6/28	13/6/26	15/6/26	15/6/26
Tide		E 2	F 3	F 3	F 1	F 2	F 4	F 6	F 6	F 1	F 5	F 2	F 5	F 6	F 6	F 6	F 6
Depth		Chlorine, grams per litre															
Yards	Metres																
0	0	16.79	16.00	15.93	16.00	15.75	13.80	14.80	14.60	12.81	13.68	12.68	12.62	14.00	16.38	16.42	16.52
2	1.8	16.79	16.10	15.95	16.05	15.83	13.86	14.89	14.66	14.08	13.74	13.81	14.63	14.10	16.44	16.41	17.07
4	3.7	16.76	16.20	15.94	16.12	16.40	13.96	15.17	14.82	14.85	15.47	13.77	15.16	14.14	16.54	16.61	17.10
6	5.5	16.85	16.30	15.90	16.13	16.37	14.12	15.62	15.10	15.18	15.49	13.77	15.73	16.50	16.51	16.59	—
10	9.1	16.88	16.36	16.03	16.40	16.54	15.37	16.09	15.83	15.53	16.08	13.89	15.80	16.50	16.64	16.59	17.16
20	18.2	16.92	16.42	—	16.48	16.58	16.33	16.59	16.18	16.27	16.31	14.97	16.40	16.65	16.65	16.71	17.17
30	27.4	16.94	—	—	16.71	16.80	16.57	16.68	16.39	16.38	16.43	16.17	16.52	—	—	16.94	17.20
50	45.7	16.98	—	—	16.85	16.85	—	16.73	16.51	16.62	16.80	16.50	16.77		—	17.18	17.31
100	91.4	17.00	—	—	17.88	17.25	—	16.99	16.97	16.80	17.09	—	—	—	17.45	17.38	17.98

TABLE XV
Chlorine, Strait of Georgia. N.E. (Fig. 2)

Station		137	142	144	110	107	104	7	10	17	40	47	48	56	61	64	70	78
Date		9/7/28	11/7/29	9/7/28	11/7/29	28/5/27	27/5/27	13/7/29	4/7/26	26/6/29	11/7/26	12/7/26	12/7/26	12/7/26	12/7/26	12/7/26	12/7/26	15/6/26
Tide		F 6	E 3	E 3	F 3	F 2	E 2	F 4	F 6	F 1	F 1	F 2	E 1	E 2	F 1	F 5	F 6	F 3
Depth		Chlorine, grams per litre																
Yards	Metres																	
0	0	5.35	13.14	9.88	14.71	12.16	9.54	12.68	12.61	11.11	2.64	4.86	9.84	13.62	15.39	15.20	16.01	17.14
2	1.8	8.80	14.00	—	15.32	12.18	9.46	12.78	—	13.09	—	13.79	14.25	13.67	15.41	15.67	15.91	17.14
4	3.7	10.58	15.83	—	15.53	13.78	9.73	13.09	14.38	14.01	—	14.26	14.71	14.04	15.41	15.67	16.00	17.28
6	5.5	11.68	15.90	13.55	15.78	15.27	12.61	13.65	15.29	15.40	—	14.73	14.95	14.99	15.60	15.96	16.01	17.18
10	9.1	14.53	16.10	—	16.12	15.24	16.13	15.87	16.03	15.69	—	15.06	15.81	15.82	16.06	15.89	16.08	17.29
20	18.2	15.60	16.40	—	16.52	16.22	16.58	16.00	16.32	16.48	—	15.93	16.43	16.48	—	16.44	16.48	17.28
30	27.4	15.83	16.45	—	16.74	16.50	16.71	16.70	—	16.87	—	16.30	16.51	16.79	—	16.59	16.68	17.20
50	45.7	—	—	—	—	—	16.73	—	—	17.06	—	16.50	—	—	—	—	—	17.29
100	91.4	16.78	17.15	—	17.25	—	16.76	—	—	17.22	—	17.03	—	—	—	—	—	—

The Distribution of the Surface Phytoplankton in the Strait of Georgia

Chart 4

The unit of plankton quantity used is volumes of phytoplankton in 100,000 volumes of sea water as determined by the net and centrifuge method (9).

The regions of maximum surface phytoplankton content related to the Fraser River have their respective centres 35 miles northward from the river mouth, opposite Seechelt and Departure Bay; 15 miles southward on the Boundary Bay-Mayne Island line, and westward in Trincomali Channel, especially near the passes. The northern area coincides with the chlorinity area 10.5 to 13.5 gm. Cl while the southern and western regions are found where the chlorinity is between 14.5 and 16.5 gm. Cl. The chlorinity may not be a limiting factor directly but these regions are characterized in each case by the most complete mixing of sea water and river water and evidently each contributes some essential condition or factor necessary for the rapid growth of phytoplankton. Near the Fraser River mouth the surface plankton cannot be measured by the centrifuge method while in the maximal areas the quantity may exceed 100 volumes in 100,000 of sea water as determined by the centrifuge method, or a litre of compacted diatoms from a cubic meter of sea water. According to the light conditions, instruments can be seen to a depth of one or two yards only. The phytoplankton area north of the river contains species of *Chaetoceros* chiefly during the summer months and those neighboring Boundary Bay and in Trincomali Channels have in addition a large *Sceletonema* proportion. The smaller rivers have correspondingly small areas of abundant phytoplankton near their mouths. The areas of maximal plankton are near the small rivers to which they are related presumably because optimal mixing is obtained at a short distance. Also, if the mixing area is restricted, as in the cases of Baynes Channel and Stuart Channel there is an abundant flora, while a more extended phytoplankton area results in less density, provided the river water supply is of the same order of magnitude, as in the cases of Bute Inlet, Sutil Channel and the northeastern portion of the Strait of Georgia.

Isohalide Vertical Section

(*a*) *Haro Strait, Boundary Pass, Southwest Strait of Georgia and Discovery Passage (Fig. 1, Table XIV)*

(*b*) *Rosario Strait, Northeast Strait of Georgia, Sutil Channel and Bute Inlet (Fig. 2, Table XV).*

In so far as is possible the data illustrated are for the months of July and August and were taken on the flood tide. Fig. 1 and 2 were drawn from the data recorded in Tables XIV and XV.

Fig. 1 and 2 show that the region under consideration is a great basin and that to a depth of 30 yards (27.5 metres) and in many places to 50 yards (45.7 metres) the Strait of Georgia has a chlorinity lower than the surface water either in the Strait of Juan de Fuca or in Johnston Strait, during the months of July and August, as is indicated by the isohalide line, 16.5 gm. Cl.

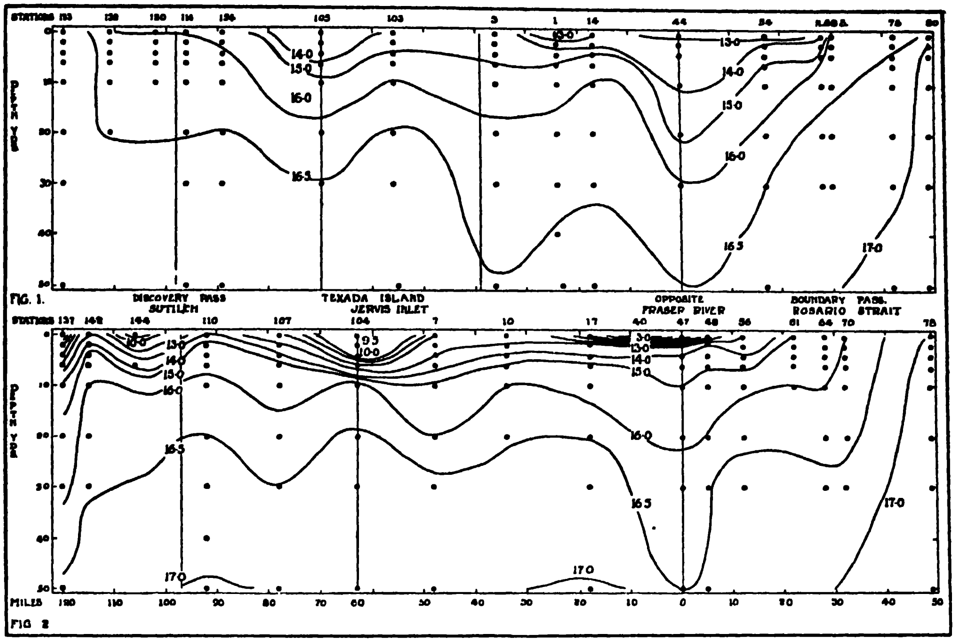


FIG. 1 AND 2. *Salinity as chloride, Strait of Georgia.*

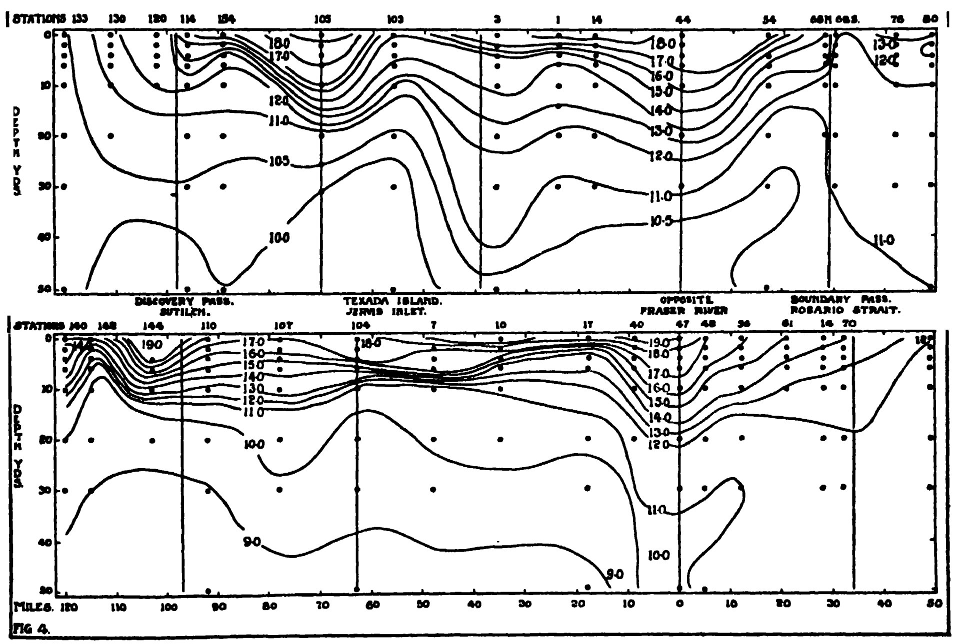


FIG. 3 AND 4. *Temperature, Strait of Georgia*

The line 16.0 gm. Cl has an average depth of 15 yards (13.7 metres); the line 15.0 gm. Cl extends the full length of the Strait, 130 miles, except near the entrance of Discovery Passage and ordinarily attains a depth varying from 5 to 10 yards (4.5-9.0 metres); the line, 14.0 gm. Cl, extends the complete distance from 18 miles south of the Fraser River on the northeastern side of the Strait of Georgia to its northern limit and onward through Sutil Channel to Bute Inlet, with the possible exception of a small area near Jervis Inlet; its most extreme depths are seven yards near the mouth of Jervis Inlet and nine yards near the mouth of Bute Inlet. On the southwestern side the line, 14.0 gm. Cl, is limited to the region from 25 miles south of the Fraser River to 30 miles north, and to that region westward from the north end of Texada Island which is affected by the waters from Bute, Toba, and Jervis Inlets, and is at the region of their fusion. It may be noted that the depth of the last-mentioned region is five yards (4.5 metres) while that at the Fraser River mouth is two yards (1.8 metres); on the other hand the depth at a point opposite the Fraser River, 15 miles westward, is 11 yards (10 metres). It is evident that the depth to which the effect extends depends upon at least two factors, namely, the extent of the water source and the distance from the source. On this basis may be explained the fact that although the northeastern side of the Strait of Georgia receives a greater supply of river water and has a proportionately low surface and near surface salinity in general (Fig. 2), still, the southwestern side shows a lower salinity at the depths between 30 and 50 yards (27.4 to 45.7 metres), Fig. 2.

It may be noted that Rosario and Haro Straits have a greater salinity than Discovery Passage, as indicated by the line 17 gm. Cl.

Region Dominated by the Fraser River

This region is characterized by a decided lowering of the salinity in a region which extends directly across the Strait of Georgia; moreover, the salinities from 14.0 to 16.5 gm. Cl attain a greater depth at station 44, 15 miles west of the Fraser River mouth, than at the lightship, at the limit of the river's delta. Similarly this region extends from north to south for a distance somewhat greater than 20 miles. An east-to-west vertical section of this area is described later. The rapid change in salinity, especially near the surface, at Boundary Pass is evident from the chart, (Fig. 1, station 68), and this may be contrasted with the gradual transition northward from the Fraser River.

Region Dominated by Jervis Inlet

The region dominated by Jervis Inlet waters at the mouth is most evident near the surface and results in a chlorinity of 10 gm. Cl to a depth of 5 yards (4.5 metres) and is noticeable to 8 yards (7.3 metres) where the chlorinity is 15.0 gm. Cl. Below 10 yards (9.1 metres), on the contrary, the salinity is greater than at either station 107, 17 miles north, or station 7, 13 miles south. In fact, it appears that the waters from Jervis Inlet affect the salinity at a greater depth northward and southward, that is, from depths of 10 to 30 yards (9.1 to 27.4 metres) at the stations mentioned, situated at the north and south

ends respectively of Malaspina Strait, than at the mouth of Jervis Inlet itself (Fig. 2). Moreover, it appears that the low chlorinity water has its effect to depths between 10 and 50 yards (9.1 and 45.7 metres) at station 3; that is, at the region westward from the south end of Texada Island (Fig. 1), and it may also flow around the north end of Texada Island at the lower depths and combine with the waters from Bute and Toba Inlets to produce low salinity water at station 105 (Fig. 1). In this instance as in that of the Fraser the effect reaches its greatest depth near its distance limit. The chlorinities between 10 and 30 yards (9.1 and 27.4 metres) depth at station 105 are similar to those at station 107. The chlorinities at station 103 are generally the highest of any in the Strait, in contrast with the low chlorinities at stations 3 and 105. The two latter are influenced by waters from Jervis Inlet; the former, although nearer, is separated by Texada Island.

Region Dominated by Bute Inlet

This region extends beyond 50 yards (45.7 metres) depth at the mouth of the inlet as indicated by the line 16.5 gm. Cl (Fig. 2). At Savary Island (station 110) the effect of the eastward drift from Discovery Passage modifies the chlorinity, while the river water reaches the lower depth of 30 yards (27.4 metres) at the north end of Texada Island, station 107. The combined effects of the rivers from Jervis, Bute and Toba Inlets reinforce the local stream, Powell River. To determine the relative values of these rivers as sources of the marked chlorinity depression between 15 and 30 yards (13.7 and 45.7 metres) depth would require more data than are available at present. The salinities at the mouth of Bute Inlet are generally lower at corresponding depths than is the case at station 45, the region of maximum depth effect of the Fraser River. This circumstance is due probably to the retention of the fresh inlet water by the passes. The increase in salinity at station 142 may be due to turbulence in the passes near this point or to the undetermined salinity of the water which issues from Toba Inlet through Calm Channel at the ebb tide.

Transition Regions—Strait of Georgia to Sea Water

Discovery Passage

Discovery Passage receives the Campbell River eight miles south of Seymour Narrows through which the tidal current often exceeds 10 knots; on the flood some river water enters also from the Sutil Channel region. The almost uniform salinity from the Oyster River mouth through Discovery Passage to station 133, and especially the small variation of 16.0 to 16.5 gm. Cl between the depths, surface to 20 yards (18.3 metres), may be explained by the complete mixing of this river water with the sea water (Fig. 2).

Southern Portion of the Strait of Georgia

This is a region beyond the Point Roberts-Saturna Island line, (stations 56 to 70, Fig. 2), where the variation between the surface and 25 yards (22.8 metres) depth is from 15.0 to 16.0 gm. Cl except at the surface near station 56. In this transition region between the sea water and the area dominated by the river the most abundant phytoplankton is located.

TABLE XVI

TEMPERATURE, STRAIT OF GEORGIA, S.W. (FIG. 3)

Station		133	130	120	114	154	105	103	3	1	14	44	54	682	685	76	80
Date		9/7/29	8/7/28	7/7/28	9/7/29	9/7/29	6/7/28	28/5/28	3/7/26	4/7/26	10/7/26	30/6/27	30/6/26	13/6/28	13/7/26	15/6/26	15/6/26
Tide		E 2	F 3	F 3	F 3	F 1	F 2	F 4	F 6	F 6	F 1	F 5	F 2	F 5	F 6	F 6	F 6
Depth		Temperature, °C.															
Yards	Metres																
0	0	10.45	11.82	12.40	15.20	16.21	18.1	14.90	17.00	19.0	18.30	17.53	17.20	14.00	12.10	13.40	13.0
2	1.8	10.40	11.79	12.55	14.95	14.88	17.97	14.73	16.99	17.52	17.53	17.62	15.27	13.55	11.95	12.55	11.75
4	3.7	10.38	11.70	12.48	14.78	12.18	18.08	14.37	15.08	15.10	15.90	17.40	14.60	13.44	11.73	12.25	11.80
6	5.5	10.20	11.42	12.19	13.99	12.03	17.96	13.37	15.55	13.82	14.86	17.23	13.38	13.18	—	12.10	12.35
10	9.1	10.19	11.09	12.03	12.01	11.37	17.90	11.34	14.30	13.20	13.62	16.42	12.62	11.68	11.70	12.00	12.00
20	18.2	10.18	11.03	—	11.81	10.87	10.85	10.20	12.32	11.13	11.64	13.82	10.75	10.92	11.60	11.80	11.62
30	27.4	10.15	—	—	10.49	10.38	10.02	9.75	11.53	10.58	11.01	11.21	10.10	—	11.48	11.38	11.57
50	45.7	10.15	—	—	9.76	10.02	—	9.33	10.38	—	—	—	10.92	—	—	10.95	11.08
100	91.4	10.00	—	—	8.73	8.45	—	8.40	9.28	9.40	—	—	9 66	—	9.95	9.70	9.40

TABLE XVII

TEMPERATURE, STRAIT OF GEORGIA, N.E. (FIG. 4)

Station		140	142	144	110	107	104	7	16	17	40	47	48	56	61	64	70	73
Date		9/7/28	11/7/29	9/7/28	11/7/29	28/5/27	27/5/27	13/7/29	4/7/26	26/6/29	11/7/26	12/7/26	12/7/26	12/7/26	12/7/26	12/7/26	12/7/26	15/6/26
Tide		F 6	E 3	E 3	F 3	F 2	E 2	F 4	F 6	F 1	F 1	F 2	E 1	E 2	F 1	F 5	F 6	F 3
Depth		Temperature, °C.																
Yards	Metres																	
0	0	14.0	14.42	19.0	18.00	16.22	17.80	19.00	19.00	18.40	19.00	18.30	17.00	16.3	14.9	14.5	13.50	12.40
2	1.8	13.65	13.95	—	16.23	16.37	17.56	17.21	—	14.97	—	17.86	16.50	15.78	14.41	13.94	13.28	11.75
4	3.7	14.17	10.39	—	15.63	15.43	17.34	16.97	15.73	14.76	—	17.97	16.02	15.56	14.18	13.69	13.09	11.75
6	5.5	13.32	10.20	19.0	14.36	14.55	14.35	16.57	13.60	13.20	—	17.22	16.00	14.93	13.66	13.92	13.00	11.38
10	9.1	12.28	9.41	—	12.40	13.26	10.35	10.72	11.60	12.19	—	16.62	13.41	14.16	12.70	13.92	12.92	11.38
20	18.2	9.94	9.06	—	9.68	10.75	9.60	10.62	10.75	10.18	—	12.60	11.33	11.33	—	11.82	11.96	11.38
30	27.4	9.52	9.00	—	9.02	9.98	2.94	9.88	—	9.60	—	11.37	—	11.07	—	11.61	11.48	11.18
50	45.7	—	—	—	8.22	—	8.81	—	—	8.96	—	10.92	—	—	—	—	—	11.18
100	91.4	8.75	8.38	—	7.86	—	8.38	8.65	—	—	—	10.18	—	—	—	—	—	—

Region Dominated by Waters from the Sea

The region dominated by waters from the sea entering through Juan de Fuca Strait and Johnston Strait may be taken as having a salinity greater than 16.5 gm. Cl. This includes the Strait of Georgia below the depth of 50 yards (45.7 metres) and in many places that below 30 yards (27.4 metres). There is a rapid depression of the transition region from the surface between stations 76 and 80 in Haro Strait to the 50 yard (45.7 metre) level at station 44, opposite the Fraser River. At the north, the effect of the sea predominates below the 30 yard (27.4 metre) level southward to Lasqueti Island on the west and to Burrard Inlet on the east side of the Strait.

Isotherm Vertical Section

(*a*) *Haro Strait, Southwest Strait of Georgia and Discovery Passage (Fig. 3, Table XVI)*

(*b*) *Rosario Strait, Northeast Strait of Georgia, Sutil Channel and Bute Inlet (Fig. 4, Table XVII)*

In the regions dominated by river water there is a remarkable parallelism between the general configuration of the isotherms and the isohalines. Among the most significant parallelisms are:— (1) The increased temperature of the regions dominated by the Fraser River, particularly at stations 47 and 44; by the waters from Jervis Inlet at station 104, near the surface, and stations 3, 105 and 107 at greater depths; by the Bute and Toba Inlet waters at stations 137 and 144 near the surface, and at stations 137, 110 and 107 at greater depths. (2) The more gradual decrease of temperature with increased depth on the southwest side of the Strait as compared with the northeast side explained by the descent of the warmed, less saline waters as the distance from river sources is increased. (3) There is a pronounced parallelism between the isotherm, 12° C., and the isohaline, 16 gm. Cl, which is of sufficient exactness to place it within the limits of an experimental error.

This parallelism does not exist to the same extent at the surface or in the regions which are dominated by sea water. Between five yards (4.6 metres) and the lower limit of river dominance the controlling factor in the temperature control is the proportion of warmer river water and of colder sea water, which chiefly is expressed by the chlorinity. The layer of river water proper is of such stability that at the mouth of the Fraser River it is found in the upper four yards (3.6 metres); here the chlorinity at 4 yards (3.6 metres) is 13.0 gm. Cl. The river water which penetrates beyond this level has remained at the surface for a time sufficient to be warmed to a temperature which is practically uniform. The heating effect is correspondingly constant. The temperature of the surface water, on the other hand, is an expression involving a number of factors, chiefly: (1) the temperature of the river at its mouth; (2) the amount of insolation, which depends upon solar intensity and time, (the time is greater at greater distances from the source); and (3) the proportion of the river and sea water (which may be determined from the chlorinity). The great differences between the temperatures at the mouth of the Fraser

River, near the head of Howe Sound, at the entrance of Powell River and in Bute Inlet, locations where the salinities are similar, may be explained on the basis of the temperature of the source and the time of exposure to radiation.

The isotherm vertical section within the *region dominated by the sea water* has a number of important features:— (1) The sea water which enters Discovery Passage, station 133, has a temperature of 10.45° C. at the surface and 10.15° C. at 50 yards (45.7 metres) depth. (2) The sea water entering at Haro Strait and Rosario Strait has a temperature which ordinarily is 12.40° C. at the surface and 11.08° C. at 50 yards (45.7 metres) depth. (3) The temperature of the water entering the Strait of Georgia from the Strait of Juan de Fuca is 2.0° C. at the surface and 1.0° C. at 50 yards (45.7 metres) in excess of the temperature of the water entering from Johnston Strait. (4) The water at the 50-yard (45.7 metre) depth, from Burrard Inlet northward, on the northeast side of the Strait of Georgia, has a temperature 1.0° C. lower than station 133, in Discovery Passage, and 2.0° C. lower than station 80, Haro Strait, at the same level. (5) The warming effect of the sea water at the lower depths in the Strait of Georgia results in a *divergence of the isotherms from the direction of the isohalide lines in the regions dominated by sea water.* (6) In July and August the warming effect of the water entering through Haro and Rosario Straits is evident at the 50-yard (45.7 metre) level nearly as far north as the Fraser River. An earlier paper (10) drew attention to the seasonal increase in temperature and chlorinity at station 1 from May until November. This may be explained by the gradual encroachment of water from Haro Strait. The probability for the validity of this explanation is evident from the configuration of the isotherms in Fig. 6 and 7. At 50 yards (45.7 metres) depth there is a difference of 2.0° C. in 20 miles distance: at station 44 the temperature is 10.9° C.; at station 17, 10.9° C.

It would appear that the central portion of the Strait of Georgia is dominated by the water from the inlets to a depth of 50 yards (45.7 metres) at least, since the isotherms parallel the isohalide lines to this depth. *The temperatures in the Strait opposite the mouths of Jervis and Toba Inlets are relatively low and the chlorinities high, below the 10-yard (9.1 metre) level.* This would suggest that these Inlets contain a depth layer of similar characteristics which is undisturbed by the surface waters and is not dominated by the rivers. Although the inlets mentioned have not been investigated, others which are similar provide evidence in this direction. Saanich Arm at a depth of 100 yards (91.4 metres) had a temperature of 9.10° C. and a chlorinity of 17.35 gm. Cl, on July 12, 1928; Bute Inlet had a temperature of 7.64° C. and a chlorinity of 17.40 gm. Cl, on August 10, 1929. The temperatures were lower and the salinities higher than in the Strait of Georgia at the same depth. On this basis the occurrence of the inlets along the northeast side of the Strait of Georgia would explain the lower temperatures and higher chlorinities, below the 20-yard (18.3 metre) level, on the northeast as compared with southwest side of the strait.

TABLE XVIII

SALINITY AND TEMPERATURE AT VARIOUS DEPTHS (FIG. 5-16)

Fig.		5	6	7	8	9	10	11	12	13	13	14	14	15	15	15	15	16	16
Station		47	68s	137	29	44	64	110	31	7	17	68	68	52	52	17	17	7	7
Date		11/7/26	13/7/28	9/7/26	9/6/27	30/6/27	28/6/28	11/7/29	9/6/27	13/7/29	11/6/28	27/6/28	27/6/28	15/6/27	15/6/27	26/6/29	11/6/28	13/7/29	13/9/29
Tide		F 2	F 6	F 6	E 2	F 5	F 3		F 4	F 4	F 6	F 1	E 2	F 6	E 6	E 6	F 6	F 4	E 4
Depth																			
Yards	Metres	Chlorinity in grams chlorine per litre																	
0	0	4.86	16.38	5.35	0.04	12.68	13.44	14.71	4.34	12.68	8.34	14.34	13.97	16.90	7.87	11.11	8.34	12.68	13.28
2	1.8	13.79	16.44	8.80	0.07	13.81	13.78	15.32	7.20	12.78	8.95	16.30	14.32	16.70	9.01	13.09	8.95	12.78	13.78
4	3.7	14.26	16.54	10.58	13.41	13.77	14.12	15.53	14.16	13.09	9.60	16.50	14.39	16.76	11.49	14.01	9.60	13.09	14.11
6	5.5	14.73	—	11.68	14.90	13.77	14.07	15.78	14.60	13.63	11.24	16.77	14.47	16.74	13.10	15.40	11.24	13.63	14.23
10	9.1	15.06	16.51	14.53	15.61	13.89	14.77	16.12	15.34	15.87	14.04	16.77	14.47	16.73	14.47	15.69	14.04	15.87	15.38
20	18.2	15.93	16.64	15.60	16.33	14.97	15.07	16.52	16.50	16.60	15.75	16.80	14.90	16.89	16.48	16.48	15.75	16.60	16.60
30	27.4	16.30	16.65	15.83	16.37	16.17	15.62	16.74	16.55	16.70	—	17.00	15.45	—	—	16.87	—	16.70	—
50	45.7	16.50	17.00	16.30	—	—	—	17.00	16.80	16.90	—	17.30	—	—	—	17.06	—	16.90	—
		Temperature, °C.																	
0	0	18.30	12.10	14.0	9.50	17.53	16.50	17.45	17.00	17.63	20.5	14.8	16.00	10.50	16.70	18.40	20.50	17.63	16.90
2	1.8	17.86	11.95	13.65	9.46	17.62	15.78	16.23	16.03	17.60	19.83	11.8	15.50	10.42	16.01	14.98	19.83	17.60	16.80
4	3.7	17.97	11.73	14.17	10.59	17.40	15.36	15.63	11.59	17.31	19.17	11.3	15.30	10.34	14.54	14.76	19.17	17.31	17.00
6	5.5	17.22	—	14.32	9.96	17.23	15.35	14.36	11.03	17.01	16.17	11.00	15.17	10.36	13.32	13.20	16.17	17.01	17.10
10	9.1	16.62	11.70	12.28	9.07	16.42	14.08	12.40	10.52	12.81	13.63	10.80	15.15	10.36	12.27	12.19	13.63	12.81	14.90
20	18.2	12.60	11.60	9.94	8.65	13.82	13.33	9.68	10.10	10.31	11.04	10.70	14.15	10.28	10.37	10.18	11.04	10.31	10.31
30	27.4	11.37	11.48	9.52	8 60	11.21	12.53	9.02	9.84	9.88	—	10.68	13.13	—	—	9.60	—	9.88	—
50	45.7	10.92	10.60	9.03	—	—	—	8.60	9.40	9.40	—	10.60	—	—	—	8.96	—	9.40	—

Salinity- and Temperature-depth Curves

(*Fig. 5-16, Table XVIII*)

The configuration of the temperature- and salinity-depth curves, especially in the upper 30 yards (27.4 metres), is dependent primarily upon the sources of the water.

Fraser River Mouth

Fig. 5 illustrates the conditions *at the Fraser River mouth.* The salinity increases rapidly from the surface to a depth of two yards (1.8 metres), then gradually to a depth of 30 yards (27.4 metres). There is a gradual decrease

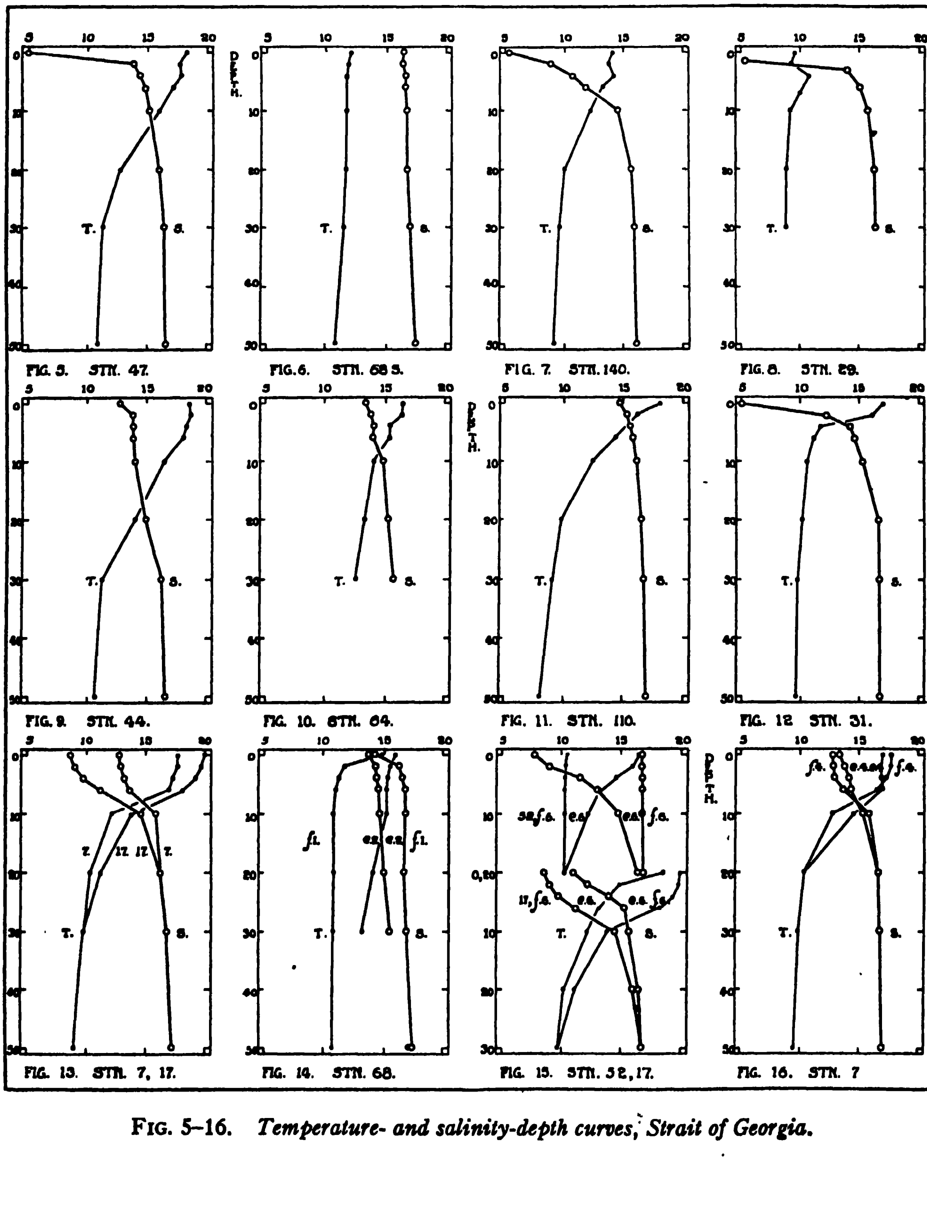


FIG. 5–16. *Temperature- and salinity-depth curves, Strait of Georgia.*

in temperature from the surface to 30 yards (27.4 metres) depth. This results in the class of configuration which may be known for convenience as the "X" type. It represents the conditions where the Fraser River is dominant and at the same time is meeting the water from the Haro and Rosario Straits. Fig. 9 represents similar conditions at station 44, which is 15 miles westward from the lightship at the river's mouth. The curves are very similar below the two-yard (1.8 metres) level. At station 64 the same type of curve is evident (Fig. 13) although modified by sea water of the type shown in Fig. 6.

Haro Strait

The conditions in Haro Strait, where the sea water dominates, are illustrated by Fig. 6. The surface has high salinity and low temperature. The change both in temperature and salinity is very uniform and gradual from the surface to a depth of 50 yards (45.7 metres). This may be known as the "H" type of configuration. It is characteristic of the waters of Haro and Rosario Straits and of Trincomali Channel at the south, and of Discovery Passage and Okisollo Channel at the north.

Regions near Rivers of Glacial Origin

These regions have salinity-temperature curves of the "n" type as illustrated in Fig. 7, 8, 11, 12. Near a river such as the Squamish there is at the surface low salinity and low temperature. The salinity increases rapidly with increased depth to four yards (3.6 metres); from this level downward there is a decrease in the rate of salinity increase. At greater distances from the river the curve maintains its general characteristics but tends to be modified toward a more uniform rate of salinity increase with depth. At greater distances from the supply (Fig. 12 and 7) of glacial water the surface temperature is increased by radiation; the near-surface decrease in temperature with depth is rapid, while toward lower depths the rate of decrease in temperature becomes less, giving graphically the upward loop of the "n" configuration. Howe Sound, (Fig. 8 and 12) and Bute Inlet (Fig. 7) illustrate these conditions.

Regions at a Considerable Distance from a River Supply

These show conditions illustrated by Fig. 15 or conforming to the "*U*" *type*. Insolation has decreased the already low specific gravity of the surface water to produce a very stable epithalassa at the upper levels, that is, to 6.0 yards (5.5 metres) depth. The decrease in temperature and the increase in chlorinity which is only slight to the depth of six yards (5.5 metres), is rapid from six yards to 10 yards (9.1 metres) and is gradual from 10 to 30 yards (9.1 to 27.4 metres) depth. Conditions at stations 7 and 17 are illustrated but the graph represents the conditions at many others, notably stations 15-20, 8-11, 3-7, 104, 105, 144, etc.

The Effect of Tides on the Configuration of the Temperature and Chlorinity Depth Curves (Fig. 14-16, Table XVIII)

At station 68, Boundary Pass, a 24-hr. series of samples was collected, July 27, 1928, with the results as shown in Fig. 14. The beginning of the ebb gave the "X" curve which is characteristic of the waters northeastward from

this station, and as illustrated in Fig. 10, while the early flood tide gave the "H" type of configuration of the waters in Haro Strait as shown in Fig. 6. The slight modifications in the upper two yards (1.8 metres) are accounted for by the fact that the samples were collected in a bay adjacent to the pass proper, and a small eddy allowed surface water to collect.

In Active Pass (station 52), which connects the Strait of Georgia, opposite the Fraser River, with Trincomali Channel another 24-hr. series was collected. As illustrated in Fig. 15 the last phase of the flood brought water from Trincomali Channel which had a temperature-chlorinity curve of the "H" type while the ebb tide gave conditions represented by the "X" curve, characteristic of the Fraser River region (cf. Fig. 6 and 9 with Fig. 15, station 52).

Fig. 15 also illustrates a change of conditions from the flood to the ebb at station 17, but not on the same day. The ebb brings water from Howe Sound which shows the "n" configuration (cf. Fig. 12) while the flood brings water from the Fraser which gives the "u" curve indicating water from a point some distance from a river supply (cf. Fig. 13).

The tidal variation is less at station 7 (Fig. 16) than at the other regions illustrated. Water from the Fraser is brought northward by the flood and that from Jervis Inlet is brought southward by the ebb. Both have the "u" shaped curve of water subjected to insolation (cf. Fig. 13); the degree of its expression is greater however in the case of the Fraser River water at the flood. It is evident from the cases cited that there is considerable mass movement of water due to the tides, and that the body of water which is transported maintains its individuality to such an extent that it may be identified at any phase of the tide by its temperature-chlorinity configuration.

Chlorinity, Temperature and Phytoplankton, Vertical Sections, on a Line Westward from the Fraser River

TABLE XIX

TEMPERATURE, STRAIT OF GEORGIA, FRASER RIVER TO PORLIER PASS AND STUART CHANNEL (FIG. 17)

Station		35	43	44	45	46	47
Date		29/7/27	29/7/27	30/7/27	6/8/26	30/7/27	29/7/27
Tide		E 6-F 1	F 2	F 5	F 4	F 4	F 3
Depth		Temperature, °C.					
Yards	Metres						
0	0	16.37	15.64	17.53	18.20	18.58	18.48
2	1.8	16.11	16.23	17.62	17.50	17.56	17.82
4	3.7	15.92	14.27	17.40	17.30	17.33	17.72
6	5.5	14.45	14.33	17.23	17.12	17.16	16.20
10	9.1	13.84	11.33	16.42	16.45	16.08	13.43
20	18.2	12.20	11.62	13.82	15.28	13.02	11.33
30	27.4	—	11.56	11.21	12.03	10.94	10.97

The data represented in Fig. 17, 18, 19, were obtained on the flood tide of two successive days, with the exception of those for station 45.

Isotherms (*Fig. 17*)

The isotherms demonstrate: (1) that the heating effect at the Fraser River mouth is limited to the upper 10 yards (9.1 metres); (2) that westward there is a rapid increase in the depth of the heating effect and that the maximum depth is reached in mid-channel; (3) that on the western side of the strait there is a decrease in the isotherm depths and as Porlier Pass is entered the temperature below 10 yards (9.1 metres) depth is less than 12° C. while at the surface it is 15.6° C.; (4) the water in Trincomali Channel, station 43, has much lower temperatures at corresponding depths than in Stuart Channel, station 35. This difference is due to the insolated river water which enters Stuart Channel from Chemainus and other rivers and which is retained by barriers.

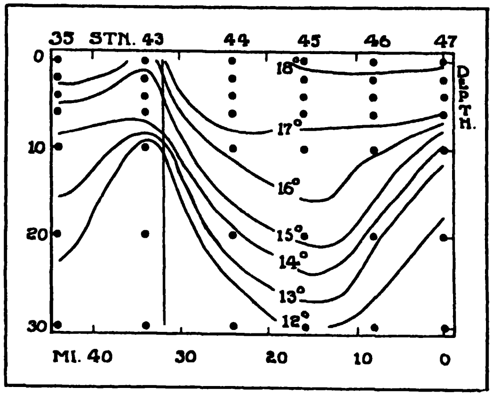


FIG. 17. *Temperature-depth curves, Strait of Georgia.*

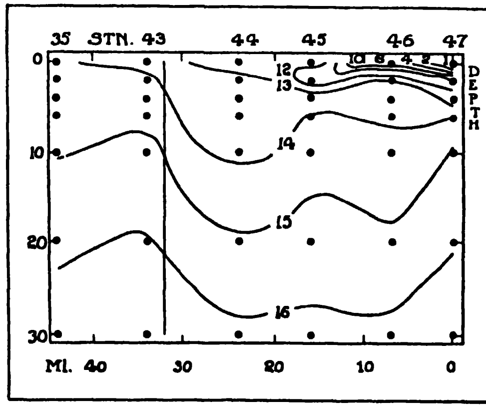


FIG. 18. *Salinity-depth curves, Strait of Georgia.*

Isohalines (*Fig. 18*)

The isohalines may be compared with the isotherms and the following features noted. (1) The relatively constant temperature, 18.58 to 17.53° C., near the surface is not entirely paralleled by the chlorinity; there are tidal waves, one of which is evident at station 45 especially from the fact that the chlorinity at two yards (1.8 metres) depth is less than that at the surface, 12.00 gm. Cl as compared with 12.52 gm. Cl. The cooling effect associated with increase in the salinity may be obviated by the greater insolation made possible by

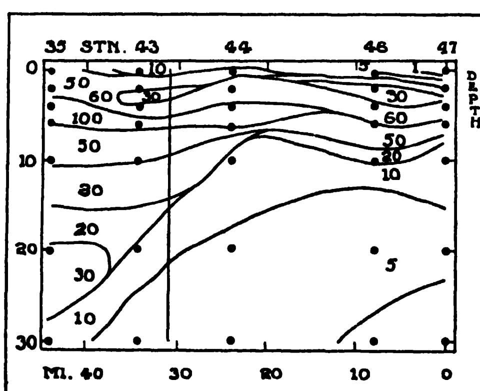


FIG. 19. *Phytoplankton-depth curves, Strait of Georgia.*

the time factor. At station 45 the near-surface temperature is higher than at station 46 although the chlorinity is greater also. Again the time factor of insolation and of mixing may account for the conditions. (2) Generally, there is a parallelism between chlorinity and temperature, that is, generally the temperature is related to the factor which expresses the degree of mixing of warmer, insolated river water and of colder sea water.

TABLE XX

SALINITY, STRAIT OF GEORGIA, FRASER RIVER TO PORLIER PASS AND STUART CHANNEL (FIG. 18)

Station		35	43	44	45	46	47
Date		29/7/27	29/7/27	30/7/27	6/8/26	30/7/27	29/7/27
Tide		E 6-F 1	F 2	F 5	F 4	F 4	F 3
Depth		Chlorinity,—grams per litre					
Yards	Metres						
0	0	14.30	13.62	12.68	15.52	5.18	0.12
2	1.8	14.34	14.30	13.81	12.00	13.69	11.31
4	3.7	14.47	14.49	13.77	13.73	13.73	12.95
6	5.5	14.78	14.74	13.77	14.38	13.80	14.02
10	9.1	14.91	15.24	13.89	14.74	14.17	15.08
20	18.2	15.68	16.11	14.97	15.23	15.18	15.90
30	27.4	—	16.12	16.17	16.22	16.22	16.57

TABLE XXI

PHYTOPLANKTON, STRAIT OF GEORGIA, FRASER RIVER TO PORLIER PASS AND STUART CHANNEL (FIG. 19)

Station		35	43	44	45	46	47
Date		29/7/27	29/7/27	30/7/27	6/8/26	30/7/27	29/7/27
Tide		E 6-F 1	F 2	F 5		F 14	F 3
Depth		Phytoplankton, volumes per 100,000 volumes of sea water					
Yards	Metres						
0	0	45	10	10	—	7	0.1
2–0	1.8–0	54	60	50	—	30	10
4–2	3.7–1.8	90	30	65	—	30	70
6–4	5.5–3.7	95	70	110	—	60	—
10–6	9.1–5.5	58	55	22	—	50	27
20–10	18.2–9.1	20	21	13	—	9	12
30–20	27.4–18.2	33	9	7	—	6	5

TABLE XXII

SALINITY AND PHYTOPLANKTON AT DEPTH (FIG. 20-27)

Fig.		20	20	21	21	22	22	23	23	24	25	25	26	26	27	27
Station		47	47	45	61	46	44	52g	64	54	52d	52d	1	10	68b	68b
Date		30/6/27	11/7/26	6/7/26	12/7/26	30/6/27	30/6/27	16/6/27	27/6/28	29/6/27	15/6/27	15/6/27	4/7/26	4/7/26	26/6/28	26/6/28
Tide		F 3	F 2	F 5	F 3	F 4	F 5	F 5	E 3	F 6	E 2	F 4	E 1	F 6	E 2	F 3
Depth																
Yards	Metres	Chlorine in grams per litre														
0	0	0.11	4.86	12.52	15.39	5.18	12.68	14.96	13.44	8.88	10.68	16.43	12.18	12.61	14.18	14.49
2	1.8	11.31	13.79	12.62	15.41	13.69	13.81	15.02	13.78	9.50	11.23	16.32	14.08	—	14.26	14.55
4	3.7	12.95	14.62	13.73	15.41	13.73	13.77	15.29	14.12	10.97	11.20	16.41	14.85	14.38	14.26	14.66
6	5.5	14.02	14.73	14.38	15.60	13.80	13.77	15.74	14.07	10.82	12.30	16.40	15.18	15.29	14.36	14.75
10	9.1	15.08	15.06	14.74	16.06	14.17	13.89	15.80	14.77	11.58	16.06	16.43	15.53	16.03	14.37	14.92
20	18.2	15.90	15.93	15.23	16.68	15.18	14.97	16.20	15.07	15.74	16.12	16.46	16.27	16.32	14.72	15.38
30	27.4	16.57	16.30	16.22	16.69	16.22	16.17	16.20	15.62	16.02	—	—	16.38	—	16.03	15.75
		Phytoplankton, volumes per 100,000 volumes of sea water														
0	0	1.2	0.5	32.0	65.0	7.5	10.0	40.0	80.0	1.5	2.5	18.0	60.0	65.0	65.0	50.0
2–0	1.8–0	10.0	—	—	75.0	30.0	50.0	38.0	40.0	25.0	10.0	14.0	—	—	90.0	30.0
4–2	3.7–1.8	—	30.0	40.0	—	30.0	65.0	15.0	35.0	16.0	8.0	—	30.0	60.0	60.0	30.0
6–4	5.5–3.7	70.0	—	—	56.0	60.0	110.0	18.0	34.0	4.0	15.0	8.0	—	10.0	28.0	15.0
10–6	9.1–5.5	27.0	22.0	8.0	10.0	50.0	22.0	9.0	22.0	105.0	5.0	—	8.0	—	35.0	12.0
20–10	18.2–9.1	12.0	10.0	4.0	4.0	9.0	13.0	3.0	12.0	22.0	2.0	5.0	—	2.0	20.0	17.0
30–20	27.4–18.2	5.0	5.0	3.0	3.0	6.0	7.0	3.0	7.0	15.0	1.0	—	0.5	—	20.0	14.0

Phytoplankton

The vertical section which shows the phytoplankton regions according to quantity demonstrates the following. (1) That the surface phytoplankton near the river mouth, station 47, is minimal and that it increases toward station 35, in other words, there is an increase in phytoplankton with increase of chlorinity, which expresses the degree of mixing. (2) That near the river mouth the maximal phytoplankton is found between two and four yards (1.8 and 3.6 metres) depth while at stations more distant it occurs at the four- to six-yard (3.6 to 5.5 metre) level; again these levels express the regions of blending of the sea and river waters. (3) That abundant plankton is found at even greater depths in regions where mixing has been possible at such depths because of the relative instability of the column, as at stations 43 and 35. (4) That tidal waves of phytoplankton are much more marked than those of either temperature or chlorinity. In Trincomali Channel near Porlier Pass depth phytoplankton reversals occur as for instance 10, 60, 30, 70 units of phytoplankton for the regions, surface, 2-0, 4-2, 6-4 yards (1.8-0, 3.6-1.8, 5.5-3.6 metres). In conclusion, the optimum conditions for the growth of phytoplankton are not in the waters of high or of low chlorinity and not necessarily in regions of intermediate chlorinity, but rather in regions where mixing is taking place, that is, where chlorinity changes are taking place. The temperature seems to have an effect upon the occurrence of species but within the limits of summer variation found in the Strait of Georgia no direct relation between phytoplankton quantity and temperature has been observed.

Phytoplankton-chlorinity Depth Curves

(Fig. 20-27; Table XXI)

Region Dominated by the Fraser River

The region dominated by the Fraser River, Fig. 20, 22, 24, 26, representing stations 47, 46, 44, 54, 1, 10, has the following characteristics: (1) At the surface there is a chlorinity less than 10 gm. Cl and a phytoplankton quantity less than 1.0 unit. (2) At station 47 in July there is a rapid transition of chlorinity from 11.0 to 15.0 gm. Cl between the depths of 2 and 10 yards (1.8 and 9.1 metres); in this region abundant phytoplankton is present and the maximum is at 4-6 yards (3.6-5.5 metres). The transition is less rapid in August and there is less abundant phytoplankton. (3) At stations 44 and 46 the region with chlorinities from 13.0 to 15 gm. Cl occurs between the depths 2 and 20 yards (1.8 and 18.3 metres) and throughout this region there is abundant plankton, with the maximum at a depth of 6 yards (5.5 metres). The similarities in the salinity curves are accompanied by corresponding similarities in plankton curves. (4) At station 54 (Fig. 24) the chlorinity is below 10.0 gm. Cl to a depth of 2 yards (1.8 metres) and in this region there is a minimal amount of phytoplankton. The transition from 10.0 to 15.0 gm. Cl occurs between the depths 2 yards (1.8 metres) and 20 yards (18.3 metres) and again this region has abundant plankton with a maximum at 10 yards (9.1 metres). (5) The phytoplankton maximum increases in depth at greater distances

from the river; that is, at the region where the chlorinity transition reaches a lower level. (6) The quantity of phytoplankton of regions near the Fraser River is represented by a "loop" curve; the type is "sub-surface maximal".

Regions Beyond the Centre of Dominance of the Fraser River

In these regions, particularly northward, the "U" type of chlorinity-temperature curve generally maintains, but at the last of the flood the direct influence of the river is shown as an "X" curve, as shown in Fig. 26. At this phase of the tide the plankton quantity is the greatest (13). Two very similar instances are shown at stations 10 and 1. In each case the surface chlorinity is between 12.0 and 13.0 gm. Cl and, in contrast with stations where the surface chlorinity is below 10.0 gm. Cl, the maximum of phytoplankton is at the surface. There is a rapid transition between the surface and 10 yards (9.1 metres) from 12.0 to 16.0 gm. Cl and at the latter depth a minimal phytoplanktōn flora is present. Again, the region of mixing is the region of phytoplankton growth.

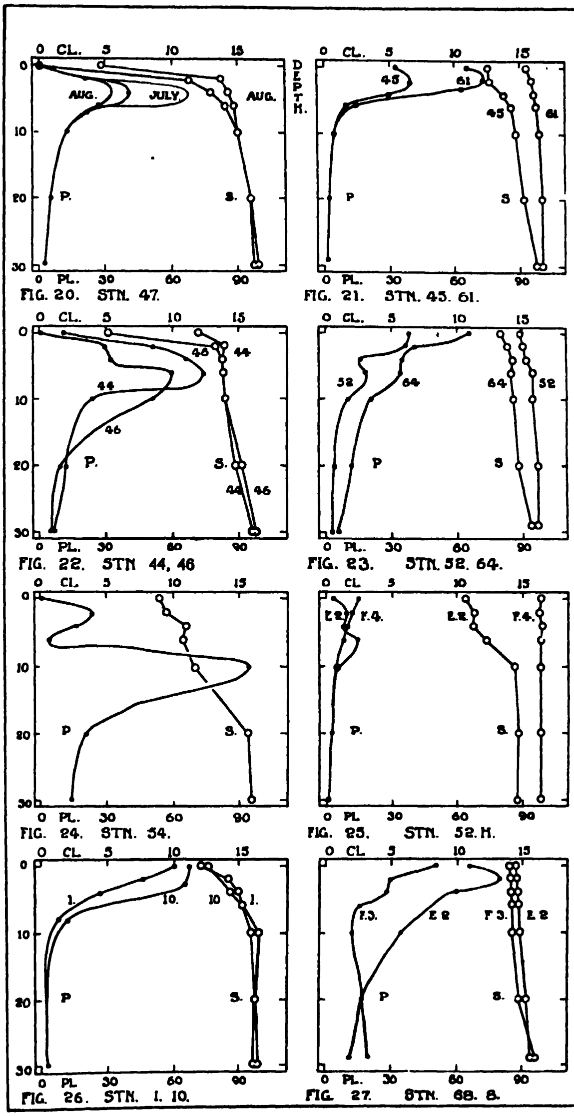


FIG. 20–27. *Phytoplankton- and salinity-depth curves, Strait of Georgia.*

At regions beyond the centre of surface dominance of the Fraser River, southward, and where the sea water dominates at lower depths, the characteristic Fraser River phytoplankton "loop" or "sub-surface maximum" curve gives place to the "diagonal" curve or "surface maximum" type.

Transition conditions between these two types are shown at stations as remote from one another as 45 and 61. At station 45, Fig. 21, the flood tide

brings water from Boundary and Porlier Passes; the surface chlorinity is 12.52 gm. Cl and at 10 yards (9.1 metres) depth it is 14.74 gm. Cl. There is a high phytoplankton quantity at the surface, 32 units, somewhat higher at 2-4 yards (1.8-3.6 metres), 40 units, and a rapid decrease to the 10 yard (9.1 metre) depth. Similarly at station 61 where the effect of the Fraser River is most marked at the last of the ebb or early flood tide. The tendency toward dominance of the sea water is shown by the salinity line approaching the "H" type in regions where the phytoplankton curve is of the "diagonal" or "surface maximum" type.

The similarity between the curves for station 52, near Active Pass in Trincomali Channel, and for station 64, north of Rosario Strait, is evident in Fig. 23. There is a gradual transition of chlorinity from the surface to the 20-yard (18.3 metre) level, accompanied by an abundant surface flora and a gradual decrease to the same level. The plankton quantity is greater in the instance which has the lower general chlorinity, that is, which is intermediate between sea and river water.

The relation of phytoplankton quantity to tides is illustrated by Fig. 22, 23, 24, 25 and 27.

At Active Pass a 24-hr. series of samples showed extremes of phytoplankton and of chlorinity at mid-ebb and mid-flood (Fig. 25). At the mid-ebb the salinity was of the Fraser River "X" type, and the phytoplankton showed the Fraser River "loop" type with a maximum at 6 yards (5.5 metres), while on the flood the water from Trincomali Channel gave the "H" chlorinity curve and the "diagonal line" phytoplankton curve with the maximum at the surface.

Another 24-hr. series at Boundary Pass gave the transition in phytoplankton types from that of Haro Strait to that of the southern portion of the Strait of Georgia (Fig. 27). The variation in salinity is very small as compared to the changes in plankton quantity which show the proportion of 1:2; other phases of the tide showed greater differences in chlorinity, for instance, at the last of the flood and the beginning of the ebb, Fig. 14. However, the greatest phytoplankton quantity occurred at mid-tide when an intermediate chlorinity between 14.0 and 15.0 gm. Cl indicated the maximal mixing of the sea and river water.

In a number of instances *tidal effects* on salinity are shown in the diagrams by *reversals* in the general trend of the curve such as between the 4- and 6-yard (3.6 and 5.5 metre) levels (Fig. 23). These indicate regions of contact of waters resulting from opposite phases of the tide, evident in the form of tidal waves which in some cases are very pronounced. At station 64 there is a decrease and at 52 an increase between the levels mentioned. These chlorinity reversals are accompanied by plankton reversals similarly of an opposite kind at the two stations. Other instances of parallel plankton and chlorinity reversals are evident in Fig. 24, and 25 for stations 54 and 52. Chlorinity reversals which are difficult to measure because they are practically within the limit of experimental error may be accompanied by a twofold or threefold

increase in plankton quantity (station 54, Fig. 24). A reversal, 10.97, 10.82, 11.58 gm. Cl, at 4, 6 and 10 yards (3.6, 5.5 and 9.1 metres) depth was accompanied by plankton quantities 16, 4, 105, at corresponding depths.

The Time Factor in Phytoplankton Quantity

From the preceding description and from the figures, it is apparent that even at the same time of the year and with similar chlorinities the phytoplankton quantity may show great variations. At the stations adjacent to the Fraser River illustrated in Fig. 20, 22, 24, the maximal plankton quantity occurs at chlorinities between 12.0 and 14.0 gm. Cl in each case. As the distance from the river increases the depth of the phytoplankton maximum increases and there is also an increase in the total phytoplankton quantity. The distance of the river's effect from its mouth, the depth to which mixing has extended and the amount of phytoplankton growth in a mixing area show parallel increases which are dependent upon the time factor. Also the most abundant surface phytoplankton is found at the most distant areas of complete mixing (Chart 4). The time factor is difficult to determine and consequently no quantitative data can be given at present. The nature of the diatom growth curve would enter into the equation. The general evidence points to the importance of the time factor and experimentation in this field should yield interesting results.

Acknowledgment

The writers wish to express their appreciation of the facilities provided by the Biological Board of Canada at the Pacific Biological Station, and by the University of British Columbia.

References

1. Anderson, F. Tide Tables, Pacific Coast of Canada, Dept. Marine and Fisheries. 1926–1930.
2. British Columbia Pilot, v. 1. Hydrographic Dept., Admiralty, London. 1923.
3. Cameron, A. T. and Mounce, I. Some physical and chemical factors influencing the distribution of marine flora and fauna in the Strait of Georgia. Contr. Can. Biol. n.s. 1: 41-70. 1922.
4. Campbell, M. H. A preliminary quantitative study of the zoöplankton in the Strait of Georgia. Trans. Roy. Soc. Can. 23, III: 1-28. 1929.
5. Clemens, W. A. Suggestions as to the standardization of plankton methods. Proc. Third Pac. Sci. Congress, Tokyo, 183-184. 1928.
6. Clemens, W. A. and Williamson, H. C. Migrations of Pacific salmon. Proc. Fourth Pac. Sci. Congress, Java, 3: 453-458. 1929.
7. Fraser, C. M. Temperature and specific gravity variations in the surface waters of Departure Bay, B.C. Contr. Can. Biol., 1918-1920: 35-48. 1921.
8. Gran, H. H. and Thompson, T. G. The diatoms and physical and chemical conditions of the sea water of the San Juan Archipelago. Publ. Puget Sound Biol. Sta. 7: 169-204. 1930.
9. Hutchinson, A. H. A bio-hydrographical investigation of the sea adjacent to the Fraser River mouth. Trans. Roy. Soc. Can. 22, V: 293-310. 1928.
10. Hutchinson, A. H., Lucas, C. C. and McPhail, M. Seasonal variations in the chemical and physical properties of the waters of the Strait of Georgia in relation to phytoplankton. Trans. Roy. Soc. Can. 23, V: 177-188. 1929.

11. Johnston, W. A. Sedimentation of the Fraser River delta. Geol. Survey of Canada, Memoir, 125. 1921.
12. Lucas, C. C. Further oceanographic studies of the sea adjacent to the Fraser River mouth. Trans. Roy. Soc. Can. 23, V: 29-68. 1929.
13. Lucas, C. C. and Hutchinson, A. H. A bio-hydrographical investigation of the sea adjacent to the Fraser River mouth. Trans. Roy. Soc. Can. 21, V: 485-520. 1927.
14. Thompson, T. G. The standardization of silver nitrate solution used in chemical studies of sea water. J. Am. Chem. Soc. 50: 681-685. 1928.
15. Thompson, T. G., Miller, R. C., Hitchings, G. H. and Todd, S. P. Studies of the sea water near the Puget Sound Biological Station during the summer 1927. Publ. Puget Sound Biol. Sta. 7: 65-100. 1929.
16. Thompson, T. G. and Van Cleve, R. Determination of the chlorinity of ocean waters. Report, International Fisheries Com. 3: 1-14. 1930.
17. Thompson, T. G. and Wright, C. C. Ionic ratios of the waters of the north Pacific ocean. J. Am. Chem. Soc. 52: 915-920. 1930.
18. Webb, C. E. Water Resources Paper No. 51. Surface water supply of Canada; Pacific Drainage; British Columbia and Yukon territory, climatic year 1924-1925. Dept. of Interior, Canada. 1927.
19. Williamson, H. C. Contr. Can. Biol. and Fisheries, n.s. 3: 265-306. 1927.
20. Williamson, H. C. Contr. Can. Biol. and Fisheries, n.s. 4: 453-470. 1929.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL of CANADA

VOLUME 5 SEPTEMBER, 1931 NUMBER 3

HEIGHT OF THE POLAR AURORA IN CANADA[1]

BY J. C. MCLENNAN[2], F.R.S., H. S. WYNNE-EDWARDS[3] AND H. J. C. IRETON[4]

Abstract

Simultaneous photographs of the polar aurora were made at two stations in northern Ontario, Canada, and from them a number of values for the height and position were obtained. For the lower limits of arcs and bands these ranged between 70 and 130 km. with a maximum frequency between 90 and 95 km. The mean height for all such lower limits was 95 km. The distribution of the points with reference to the earth's surface is shown.

Introduction

The aurora borealis has been the subject of many researches, particularly by Norwegian scientists. Professor Størmer* has made a long series of observations on the distribution, form and height of the aurora and its dependence on concomitant phenomena, and has developed the method of height measurement by simultaneous photographs on an extended base. While many of his results are of undoubted generality, it is nevertheless important that data should be obtained in as many other northerly regions as possible.

Canada, and particularly northern Ontario, is very favorably situated for such investigations. Owing to the position of the magnetic pole the zones of corresponding auroral activity lie further south there than elsewhere in the northern hemisphere, so that the aurora is commonly seen in lower latitudes than in Europe. For these reasons it was decided to make observations at a point as far north as is compatible with reasonable facilities.

Observation Stations

The main observing station was situated at Onakawana on the northern extension of the Temiskaming and Northern Ontario Railway at present under

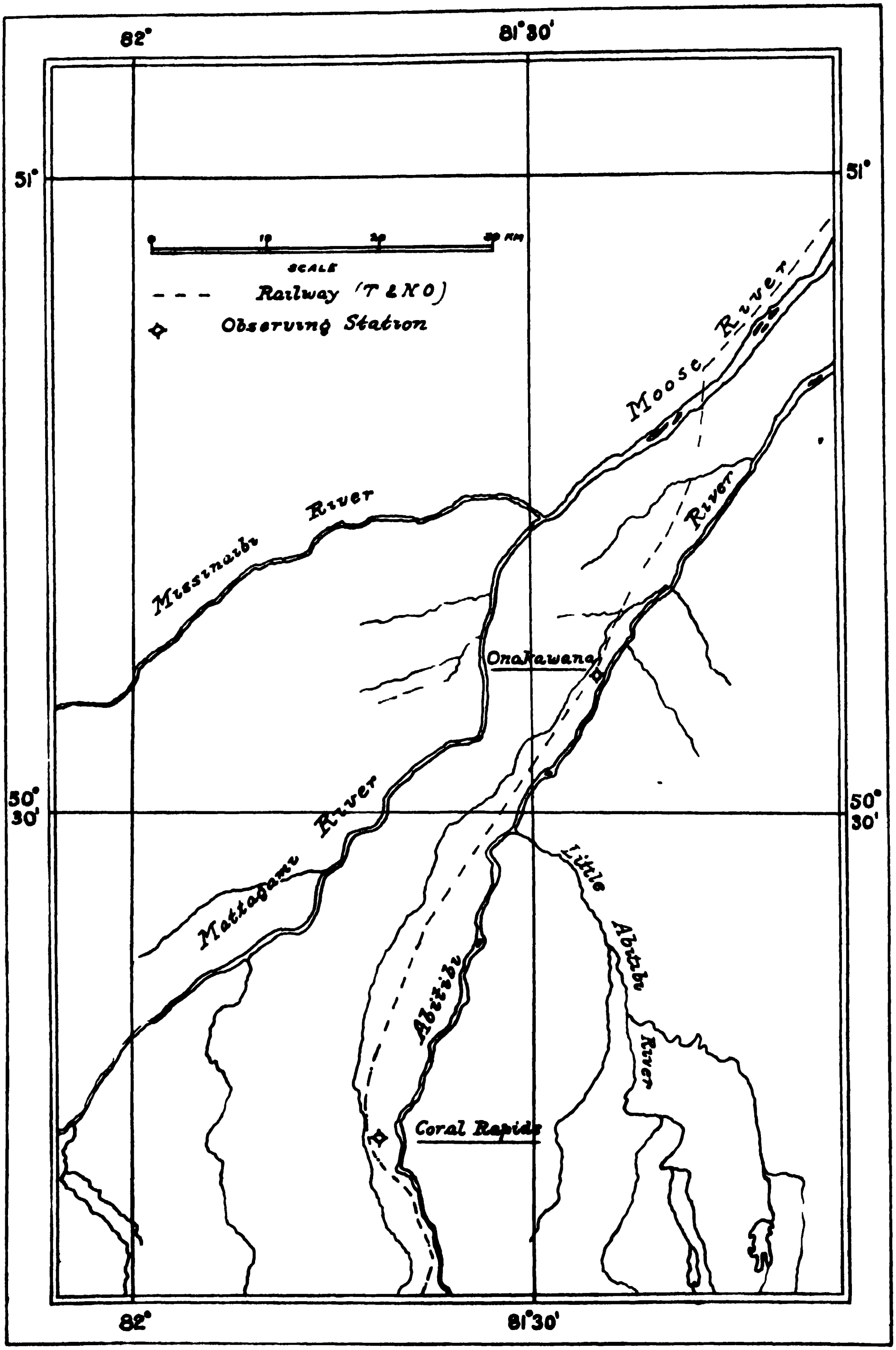
[1] *Manuscript received May 30, 1930.*
Contribution from the Physical Laboratory of the University of Toronto, Toronto, Canada, with financial assistance from the National Research Council of Canada.

[2] *Professor of Physics and Director of the Physical Laboratory, University of Toronto.*

[3] *Research Assistant, Department of Physics, University of Toronto.*

[4] *Lecturer and Research Associate, Department of Physics, University of Toronto.*

**Résultats des mesures photogrammétriques des aurores boréales observées dans la Norvège méridionale de 1911 à 1922, Geofysiske publikationer, Vol. IV, No 7, Oslo 1926, in which references to other publications may be found.*



MAP 1. *Map showing location of observation stations.*

construction, and here a small laboratory was equipped. The substation was at Coral Rapids, where photographs were taken by Mr. T. J. Kennedy, and the two stations were connected by the railway company's telephone line.

The length and azimuth of the base line were determined from the plans of the railway right-of-way, and the latitude and longitude of Coral Rapids from the survey of the Department of Lands and Forests of the Provincial Government.

Length of base line, b	$=46.40$ km.
Azimuth of Onakawana from Coral Rapids, A_0	$=$N 22°31′ E.
Latitude of Coral Rapids, ϕ	$=50°13'$ N.
Longitude of Coral Rapids, λ	$=81°40'$ W.

The heights of the two stations above sea level were approximately 50 metres for Onakawana and 110 for Coral Rapids, and the height-angle involved has been considered negligible.

Apparatus and Method of Observation

The cameras used were of a type designed by Professor O. Krogness of Tromsø, and arranged so that by moving the lens to different positions six small photographs may be taken on one 9 by 12 cm. plate. The lenses were Kino-Plasmat, D.R.P. Dr. Rudolph, made by Messrs. Hugo Meyer of Görlitz, with aperture 1.5 and focal length, 5 cm. The plates used were Herzog Sonia E W.

In order to synchronize the exposures the two observers used operator's telephones which could be worn without interfering with their movements. The time was taken from an accurate watch which was checked daily by radio signals. Since there was only one person at each station it was impossible to avoid taking the watch into the cold, but it was protected as much as possible from temperature changes.

The plates taken at Coral Rapids were sent as soon as means were available to Onakawana, and all the plates were developed there, corresponding plates being developed at the same time.

Method of Calculation

In computing the auroral heights and distances, one of each pair of simultaneous pictures was projected by a lantern on a ground glass screen, and as many stars and well-defined auroral points as possible traced on the latter. The second photograph of the pair was then so arranged in the lantern that the projected stars coincided with those already drawn on the screen, and the auroral points corresponding to those selected in the first plate were marked. In nearly every case a sufficient number of stars could be identified to admit of the insertion on the projection of the declination and right ascension circles of the celestial sphere and from this co-ordinate system a slight extrapolation enabled the declination δ and right ascension α of the auroral points to be read off. If δ_1, α_1; δ_2, α_2 are the co-ordinates for two corresponding auroral points, their angular separation, *i.e.*, the parallax of the point in question on the aurora, is given by the standard formula:

$$tg\ p = \frac{\alpha_1-\alpha_2}{\delta_1-\delta_2}\cos\frac{\delta_1+\delta_2}{2} \qquad (1)$$

which is valid for such small separations as were most frequently observed. In cases where so few stars could be distinguished as to preclude a reasonably accurate insertion of a co-ordinate system, the scale of the projected diagram in the vicinity of points of interest was calculated from pairs of stars, their angular separation being computed by (1) (or by a more accurate formula if the separation is larger), and the linear separation of the auroral points was then turned into angular measure.

From the sidereal time of exposure the right ascension of an auroral point δ, α can be expressed as an hour angle t, and by the standard equations of Gauss we may calculate the altitude h and azimuth A of the point:

$$\sin h = \sin \phi \sin \delta + \cos \phi \cos \delta \cos t \tag{2}$$

$$\sin A \cos h = \sin \delta \cos t \tag{3}$$

where ϕ is the latitude of the station. From the parallax p, the length b of the base line and its direction A_0 a simple right-angled spherical triangle alone remains to be solved to give the angle u between the base line and the line from the observer to the auroral point. We now compute the quantities

$$r = b \sin u / \sin p$$
$$x = r \cos h$$
$$y = r \sin h$$

and hence the height H of the auroral point and the distance D to the point vertically below it;

$$H = \sqrt{(R+y)^2 + x^2} - R$$
$$D \doteq \frac{R}{R+H} x$$

where R is the radius of the earth. We have neglected the height of the stations above sea level.

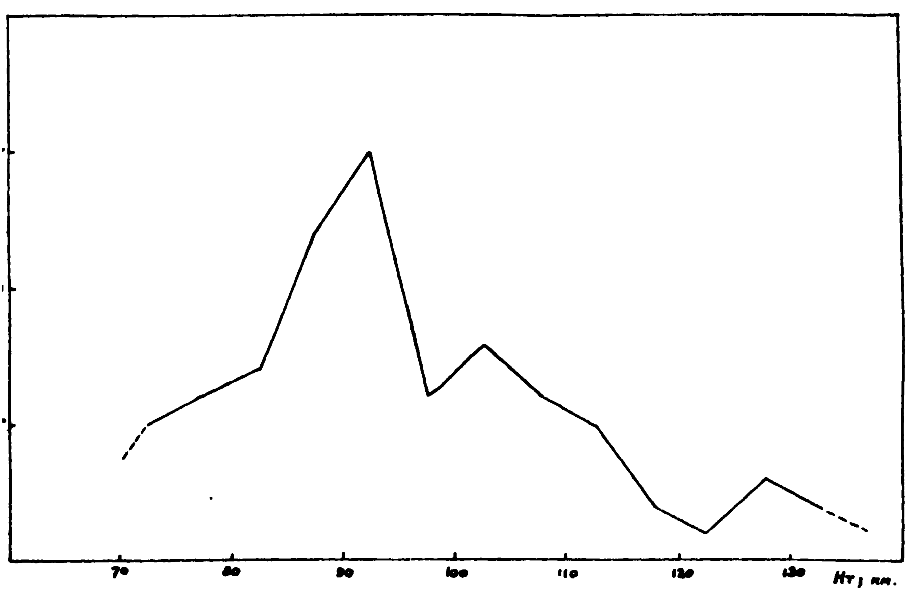


FIG. 1. *Relation between heights and frequency of occurrence.*

It will be seen that the method is essentially the simple one of Størmer used in his early work of 1912-1913. Størmer's later method involving the construction of nets to correct for distortion when a portion of a sphere is projected on a plane is unnecessary here; the smallness of h for example would hardly justify the more elaborate process. The results obtained vary in accuracy on account of several factors, the chief of which is the possibility of recognizing corresponding auroral points. In the favorable cases, however, the height will not be more than 5% in error; in about one-fifth of the cases it may be as great as 10%, and in very unfavorable cases may exceed 20%.

Results

Diagrammatic sketches of all the photographs measured are given in Fig. 2-4, in which the auroral outlines and points treated, together with their parallaxes, are qualitatively indicated. All the points lie on the lower limits

TABLE I

No.	Date 1931	Time			Exposure, sec.	Reference stars
		hr.	min.	sec.		
3	Jan. 25	23	26	30	60	α, β, η Cas, φ, ν And
4		23	30	35	60	α, η, ζ Boo, α, C Vn
5		23	33	10	30	α Dra, Θ, γ Boo, ζ, ε, U Ma
6		23	34	52	15	ι Vir, α, ε, η Boo
7		23	37	40	10	α, β, ζ, υ Cas, κ And
8		23	39	47	5	β Cas, κ, ο And, 2 Lac
9		23	41	55	27	β, ζ Cas, κ, ο And
10		23	42	30	32	κ, ο And, π^2 Cyg, ξ Cep
13		23	48	42	5	α, ο And, α Lac, β, ζ Cas
14		23	50	10	19	α, β, γ, δ, ζ Cas
16		23	54	00	51	α, γ, δ, η, μ Boo
17		23	56	10	37	α, γ, λ Boo, α C Vn
19	Jan. 26	0	02	15	8	α, γ, λ Boo, η, ζ U Ma
20		0	03	55	55	α, ε, η, τ Boo
21		0	06	20	60	ε Vir, α, ε, η Boo
22		0	18	02	25	α Cr B, β, γ, δ, ε, λ Boo
23		0	20	15	90	α Cr B, α, η Boo
24		0	24	11	48	β, γ, δ, ν, π, ξ Dra
25		0	27	15	57	β, γ, ν, ξ Dra, τ Her
27		0	31	15	35	41 Ari, β Tri, β, γ And
28		0	32	38	25	β, ν, ξ Dra, τ Her
29		0	33	10	40	β, γ, ν, ζ Dra, τ Her
30		0	35	50	30	α, λ, ξ Tau, 41 Ari, ζ, ξ Per
31		1	11	30	80	α, β, γ, ζ Cas
32		1	13	05	17	α, β, γ, ζ Cas
33		1	13	30	10	41, 35 Ari, γ, β Tri
34		1	16	17	4	η Tau, β, ζ Per
36		1	21	05	48	β, γ, ε Boo, γ, τ, φ Her
37	Jan. 27	21	39	19	30	β, η Peg, ο And, α, π^2 Cyg
38		21	41	41	40	ε, η, ζ U Ma, α C Vn, γ Boo
39		21	44	43	33	β, γ, ν, ξ, η, φ, Dra, β, γ U Ma
40		21	49	47	24	α Cep, α Cyg, α Lac
41		21	52	04	18	α Cyg, α, 4 Lac, α, β Cep
43	Feb. 13	22	22	05	19	γ, β, ξ, ν, η Dra, τ Her
44		22	23	50	44	τ Her, γ, β, ν, ξ, 39 Dra
45		22	26	17	5	β, γ, ν, ζ, ξ, η, Θ Dra
46		22	27	40	65	α Cyg, α, β Cep, δ Dra
47		22	28	30	14	α, β, γ, ζ Cas, γ And
48		22	30	36	6	α, β, γ, ζ Cas

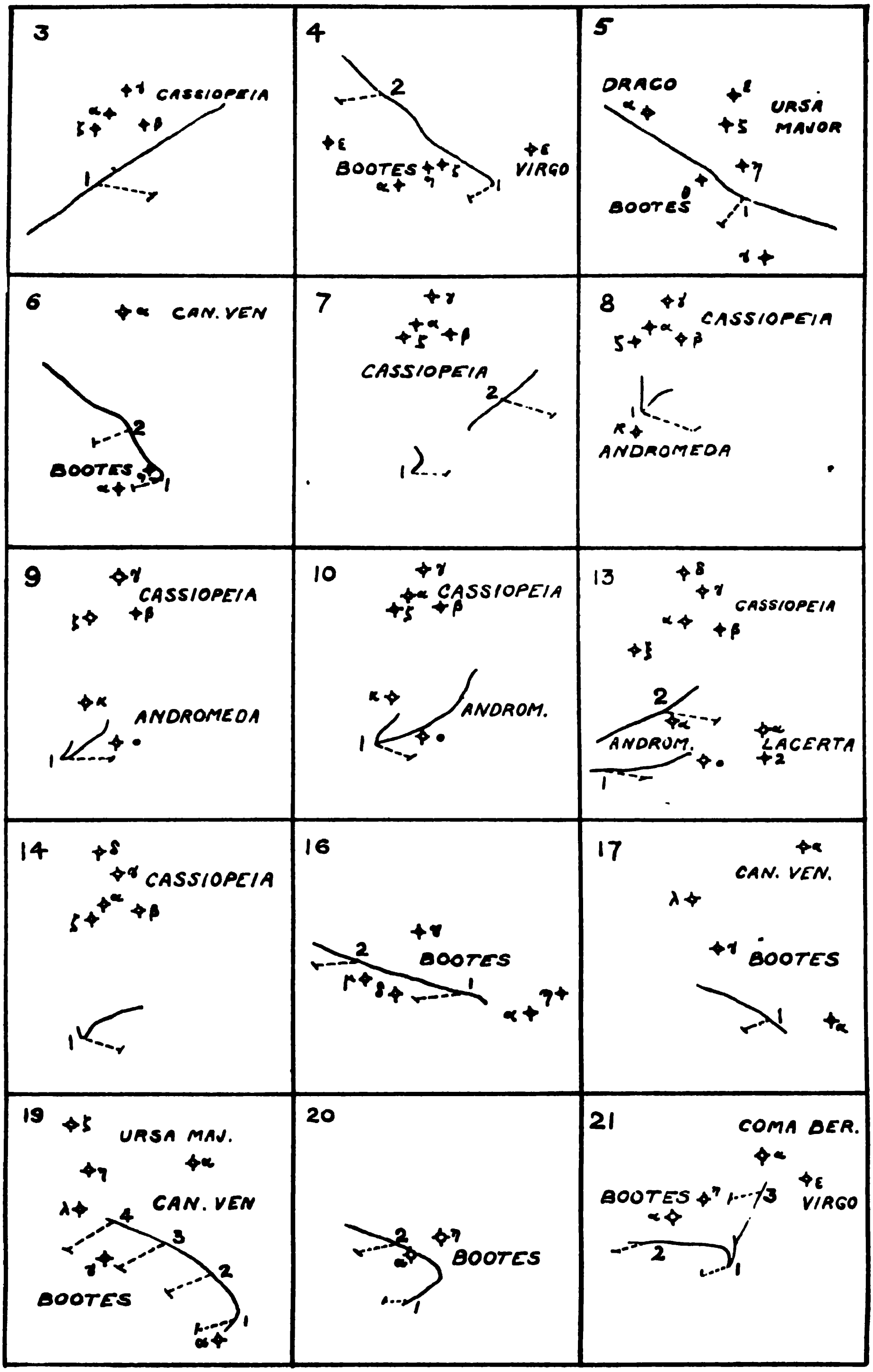


FIG. 2. *Sketches of photographs measured: No. 3-17 taken Jan. 25, 1931; No. 19-21 taken Jan. 26, 1931.*

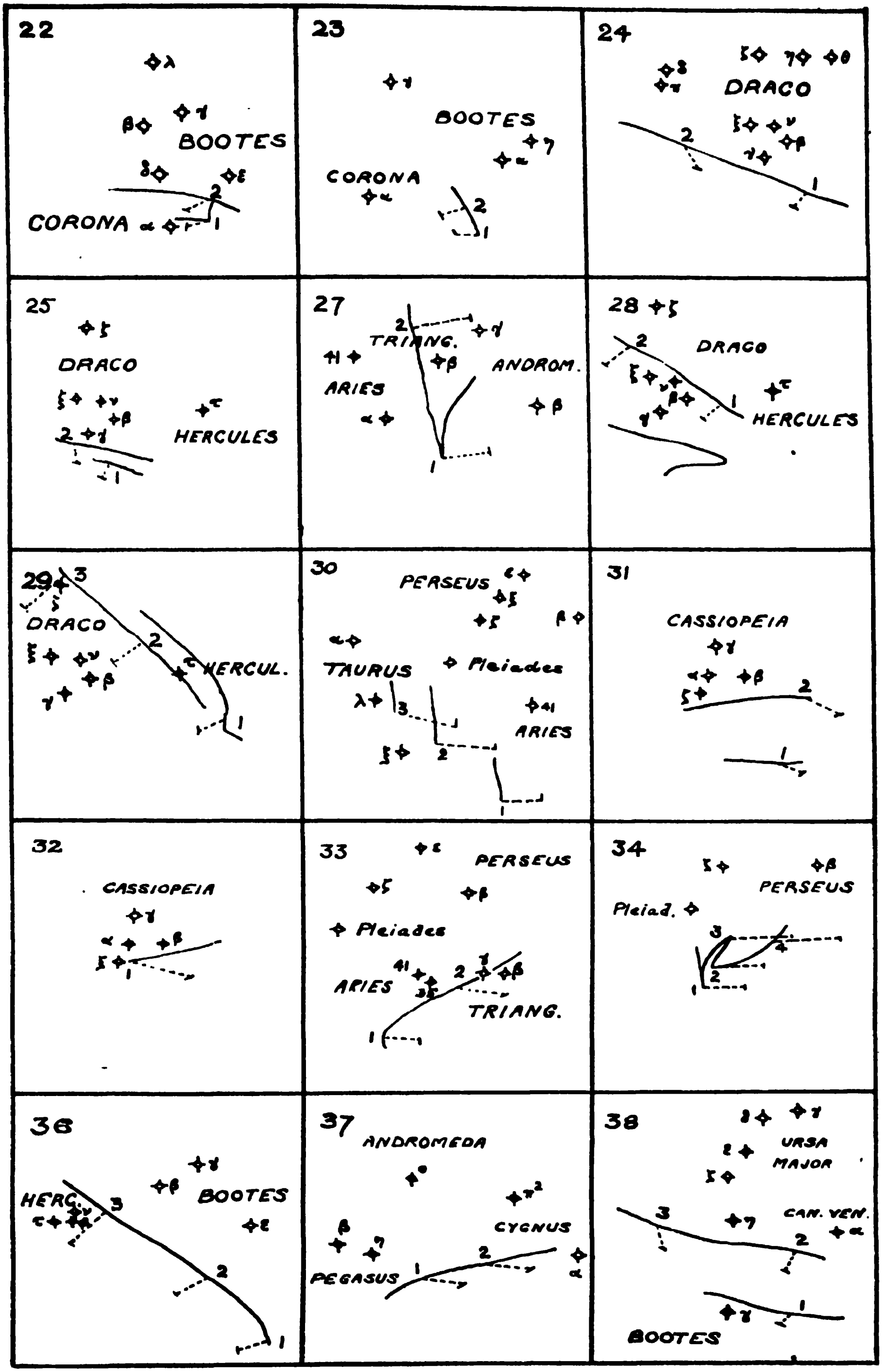


FIG. 3. *Sketches of photographs measured: No. 22-36 taken Jan. 26, 1931; No. 37 and 38 taken Jan. 27, 1931.*

of bands with the exception of No. 21,3; 27,2 and 48,2 which lie on streamers, and of No. 39,5 which is on the upper limit of a band.

In Table I are given the 75th meridian time at the middle of the exposure and the length of exposure. The fifth column shows the principal reference stars used in the measurements.

The results of the measurements are shown in Table II. In it are tabulated the parallactic angle p of the auroral point, α, δ, A and h, its right ascension, declination, azimuth and altitude with reference to Coral Rapids, and H and D the height and distance of the point.

The heights have been plotted against frequency of occurrence in Fig. 1, those heights lying in ranges less than 70, from 70 to 75, 75 to 80, ..., km. being collected into groups.

The distribution of the auroral points in relation to the earth's surface has been indicated on Map II. Points on the same definite band have been joined, isolated small circles showing single observations.

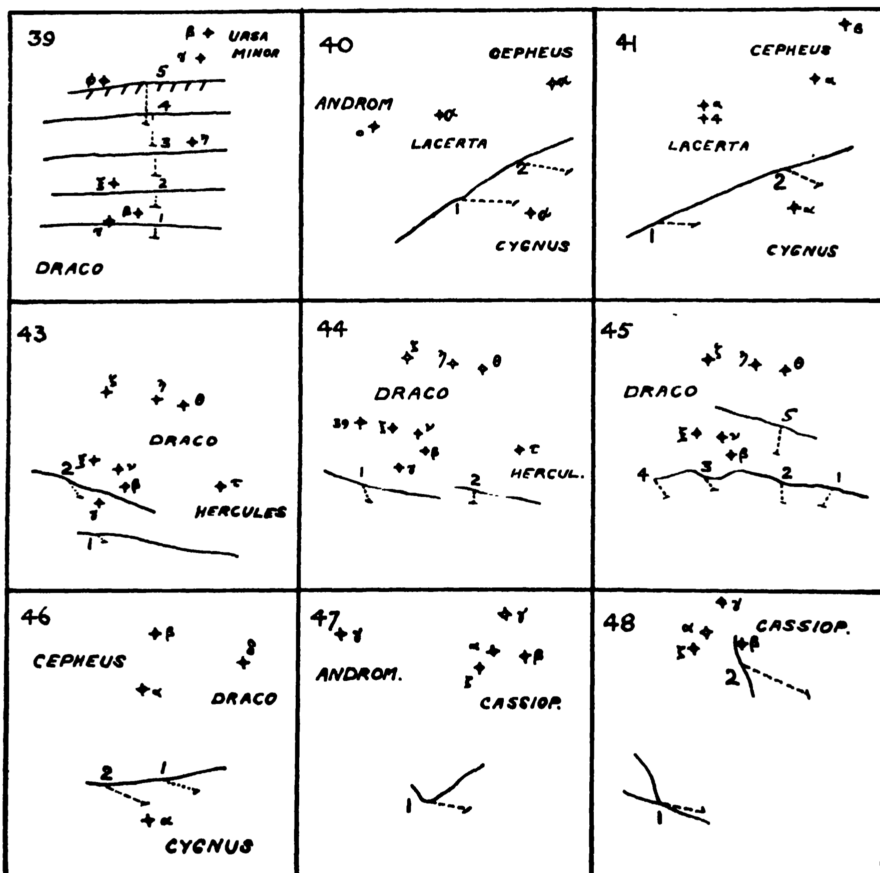
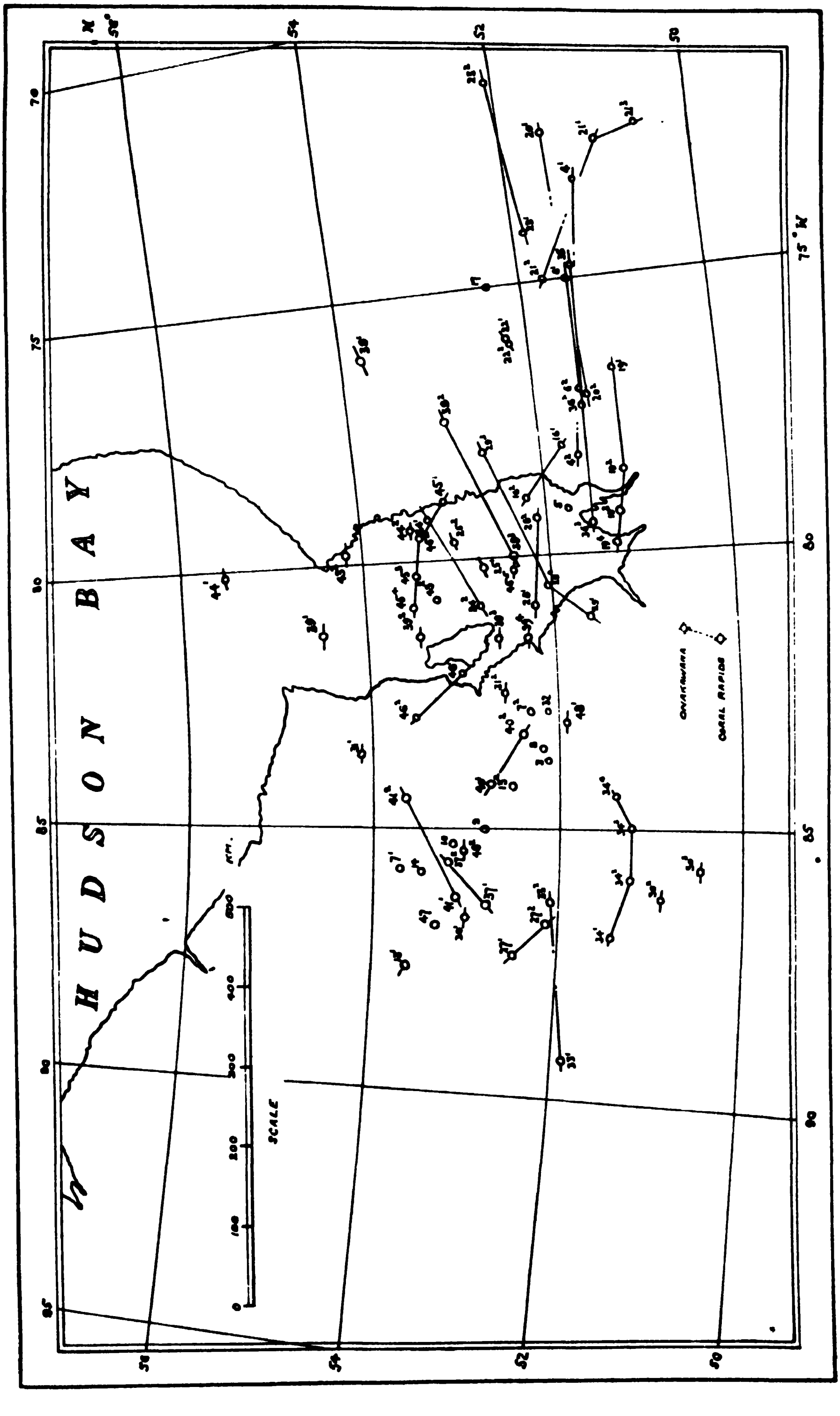


FIG. 4. *Sketches of photographs measured: No. 39-41 taken Jan. 27, 1931; No. 43-48 taken Feb. 13, 1931.*



MAP 2. *Distribution of auroral points in relation to the earth's surface.*

TABLE II

No.	Date 1931	Time			P	p	α		δ	$A-180°$	h	H	D
		hr.	min.	sec.		°	hr.	min.	°	°	°	km.	km.
3	Jan. 25	23	26	30	1	8.7	23	01	51.4	−33.8	22.1	103	239
4		23	30	35	1	3.6	13	42	12.3	78.3	6.3	96	603
					2	5.8	14	02	34.7	59.7	20.2	111	280
5		23	33	10	1	5.8	14	37	45.7	46.8	24.3	109	228
6		23	34	52	1	3.9	14	03	18.0	71.5	8.0	93	486
					2	5.1	14	01	27.0	65.9	15.2	108	354
7		23	37	40	1	4.6	22	41	41.5	−34.5	10.9	111	474
					2	7.7	21	38	56.2	−18.4	19.7	86	226
8		23	39	47	1	8.7	22	46	50.3	−30.5	18.8	85	234
9		23	41	55	1	6.2	22	58	38.3	−38.3	9.4	71	365
10		23	42	30	1	5.8	22	56	39.8	−37.0	10.4	84	390
13		23	48	42	1	4.5	23	31	33.8	−45.3	8.3	101	530
					2	7.4	23	10	47.1	−34.5	17.3	99	290
14		23	50	10	1	5.0	23	02	39.3	−37.1	9.7	93	446
16		23	54	00	1	5.1	14	56	30.4	57.3	12.7	77	304
					2	3.9	15	41	42.3	42.5	16.7	93	283
17		23	56	10	1	3.2	14	50	26.3	61.5	10.8	122	518
19	Jan. 26	0	02	15	1	5.7	14	19	17.5	74.0	9.5	71	359
					2	7.7	14	13	28.5	67.6	18.7	87	239
					3	8.2	14	26	36.4	59.7	22.6	89	202
					4	8.6	14	46	43.0	51.7	24.8	81	169
20		0	03	55	1	2.9	14	39	15.8	74.5	5.3	101	680
					2	5.3	14	32	24.8	67.0	13.3	92	344
21		0	06	20	1	3.3	14	18	10.0	79.8	4.5	88	661
					2	3.8	14	41	20.3	68.8	8.9	98	494
					3	3.3	13	32	16.2	84.4	16.5	244	674
22		0	18	02	1	3.6	15	23	25.8	59.9	8.8	86	448
					2	3.6	15	16	27.3	60.2	10.9	103	445
23		0	20	15	1	2.6	15	00	15.5	71.0	4.4	105	753
					2	3.4	15	00	18.6	69.0	6.8	93	559
24		0	24	11	1	1.3	17	43	44.2	26.8	10.1	77	368
					2	2.9	18	54	53.3	12.2	15.0	80	273
25		0	27	15	1	1.5	17	54	45.5	24.8	10.7	72	329
					2	2.0	18	08	48.6	21.4	12.7	76	285
27		0	31	15	1	5.6	1	00	27.8	−56.9	9.1	90	455
					2	5.8	2	00	38.3	−59.7	25.2	207	399
28		0	32	38	1	3.0	17	17	48.3	30.3	16.4	84	261
					2	4.6	18	32	59.9	14.8	22.5	93	213
29		0	33	10	1	2.9	16	29	38.0	43.7	12.3	92	365
					2	4.6	16	57	53.0	30.8	22.1	90	210
					3	7.6	18	00	64.4	16.8	28.0	81	146
30		0	35	50	1	5.4	2	07	16.5	−46.5	9.5	93	453
					2	7.7	2	46	18.2	−75.2	16.9	108	323
					3	8.4	3	13	17.2	−81.7	20.4	115	285
31		1	11	30	1	3.7	22	36	48.8	−15.4	11.0	104	445
					2	5.8	22	13	59.5	− 9.4	20.6	103	256
32		1	13	05	1	8.3	23	27	57.2	−19.8	21.3	88	214
33		1	13	30	1	4.7	2	21	20.0	−68.9	8.5	109	552
					2	6.7	1	57	33.2	−56.1	15.3	115	373
34		1	16	17	1	6.6	2	43	20.8	−69.6	14.5	113	386
					2	8.2	2	44	25.8	−68.7	15.8	96	308
					3	9.7	2	41	30.2	−65.0	18.7	94	257
					4	10.4	2	14	35.3	−57.0	18.8	86	226
36		1	21	05	1	4.1	15	43	16.0	74.0	7.6	87	500
					2	5.3	15	49	28.0	65.0	15.8	104	335
					3	6.7	16	18	43.4	49.5	23.5	94	207
37	Jan. 27	21	39	19	1	5.3	21	43	34.4	−47.4	10.4	94	427
					2	5.4	21	08	41.3	−37.6	12.4	107	418
38		21	41	41	1	1.8	14	01	35.0	42.7	8.1	102	545

TABLE II—*Continued*

No.	Date 1931	Time hr.	Time min.	Time sec.	P	p °	α hr.	α min.	δ °	A−180° °	h °	H km.	D km.
					2	2.6	13	36	40.4	43.8	15.3	131	418
					3	3.0	14	52	51.4	26.1	17.8	91	262
39		21	44	43	2	2.1	17	15	53.5	3.9	13.9	136	469
					3	3.1	17	13	58.0	3.8	18.3	129	352
					4	4.5	17	11	61.7	3.8	22.0	111	258
					5	5.6	17	09	66.0	3.5	26.4	114	222
40		21	49	47	1	6.8	20	58	45.7	−32.0	14.3	87	308
					2	7.5	20	25	52.1	−23.9	17.6	86	252
41		21	52	04	1	5.3	21	35	36.5	−42.6	9.8	93	445
					2	4.7	20	30	49.5	−25.5	15.3	129	414
43	Feb. 13	22	22	05	1	1.1	17	56	46.5	15.8	8.7	86	450
					2	2.2	18	12	52.8	11.7	14.3	95	332
44		22	23	50	1	1.2	18	26	49.0	10.6	10.2	134	583
					2	1.1	17	08	43.8	25.1	9.0	72	378
45		22	26	17	1	1.6	16	40	41.2	31.5	9.0	67	355
					2	1.3	17	03	44.5	26.2	10.2	79	373
					3	1.6	17	53	48.8	16.5	11.3	82	356
					4	2.3	18	32	49.7	10.0	10.8	79	357
					5	3.0	16	50	50.8	25.5	16.8	80	246
46		22	27	40	1	4.3	20	05	50.5	− 5.0	11.1	67	300
					2	4.3	20	51	48.0	−12.9	9.7	74	366
47		22	28	30	1	4.9	23	34	37.4	−44.6	12.2	126	481
48		22	30	36	1	5.7	22	59	38.3	−37.9	9.0	77	397
					2	9.5	22	42	58.9	−25.7	24.9	98	200

Discussion of Results

It has not been considered useful to attempt a definite classification of auroral types. Owing to the southerly position of the stations and the lack of displays of particular ntensity during the past winter, practically all those seen were of the arc or band type, streamers or draperies being only rarely observed. The displays followed a very regular course which it may be of interest to sketch. As soon as darkness set in a faint glow was visible in the northern sky. This was never absent on any occasion when the sky was clear. Later on a single arc might appear at a higher altitude, and as a rule was quite steady in its position. The period of maximum activity was between ten and eleven o'clock, when intense displays might flash up very suddenly, generally starting as single or multiple arcs of a pulsating type from which streamers sometimes evolved. After a time the lights lost intensity and at the same time stretched further towards the zenith, until in the early morning they might extend some way towards the south, but too dim to be photographed.

It may be observed in parenthesis that among those who have spent some time in the north there is a general conviction that there is a noise associated with brilliant northern lights, but none was noticed in the course of these observations. First-hand evidence was also received of lights occurring apparently very low down, but of a nature to preclude definite conclusions.

The height measurements obtained are in good general agreement with the prevoius work of Størmer and others in Scandinavia. For the lower limits of

bands they show a range from approximately 70 to 130 km., with a marked maximum between 90 and 95 km. (see graph). The mean height for all lower limits of bands is 95 km. This is considerably lower than in Scandinavia, where the corresponding value obtained is about 105 km. Making allowance for experimental error it appears also that the lower limit is sometimes below the lowest height reached there, which Størmer gives as 80 km.

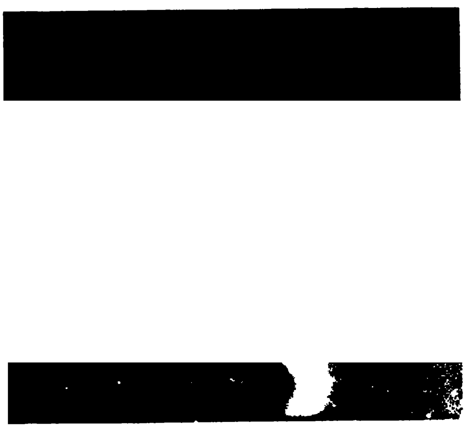
The material collected for other types of aurora is too limited to permit of any conclusions being drawn.

Acknowledgments

The authors are indebted to the Department of Mines of the Ontario Government for making it possible for one of them to spend several months at the Onakawana Coal Development fields, and to the T. and N.O. Railway for facilitating transportation and permitting the use of their telephone system. They wish to thank also the H. F. McLean Construction Company for their kindness in building the observing station at Onakawana, and the Bell Telephone Company of Canada for the loan of telephone equipment. The authors wish also to thank Mr. C. Barnes for developing a method for the rapid evaluation of results and the calculations connected therewith.

C 6

O 6



C 19

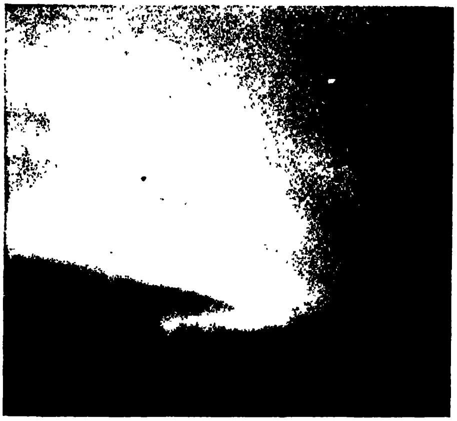
O 19

C 27

O 27

Typical simultaneous photographs of aurora taken at Coral Rapids (C) and Onakawana (O): No. 6 taken Jan. 25, 1931; No. 19 and 27 taken Jan. 26, 1931.

PLATE II

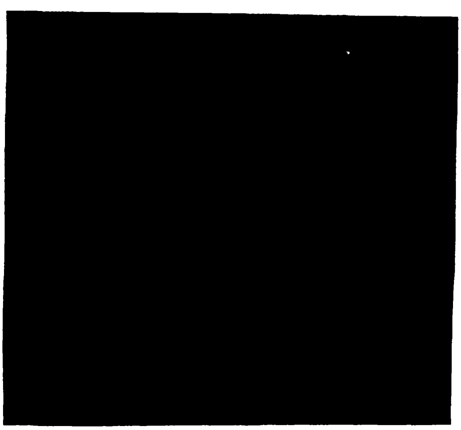


C 28

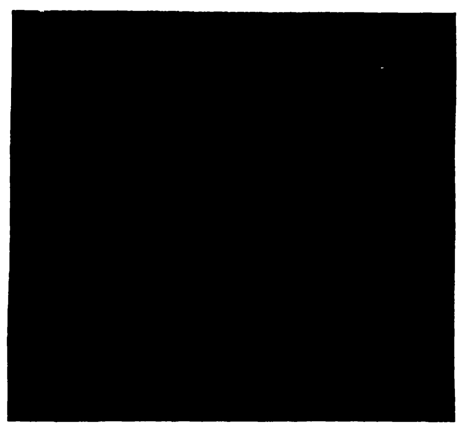
O 28

C 29

O 29

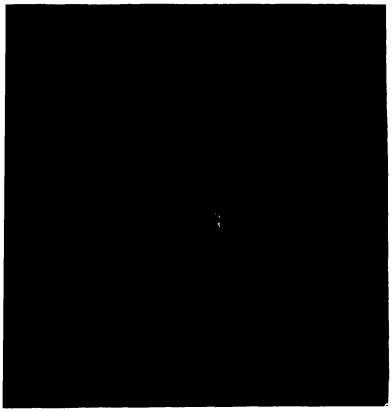


C 33

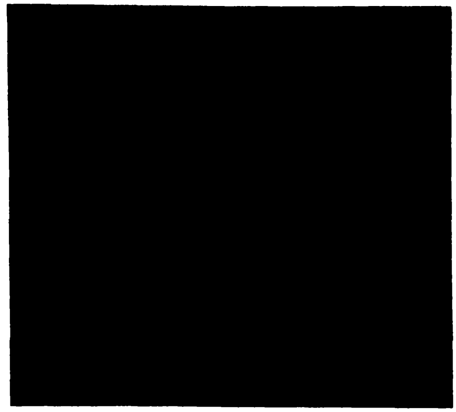


O 33

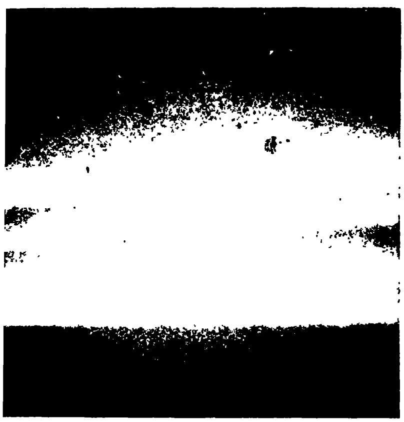
Typical simultaneous photographs of aurora taken at Coral Rapids (C) and Onakawana (O): all taken Jan. 26, 1931.



C 34



O 34



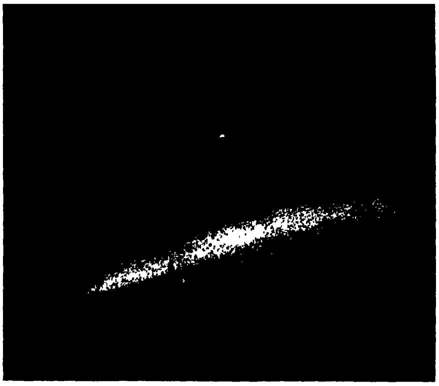
C 39

O 39

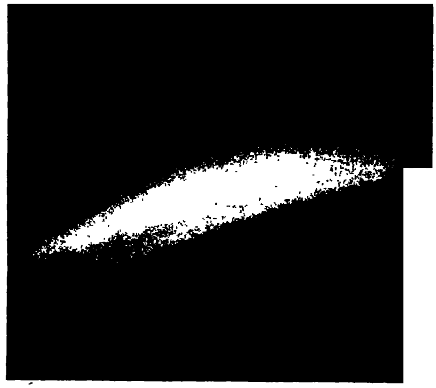
C 40

O 40

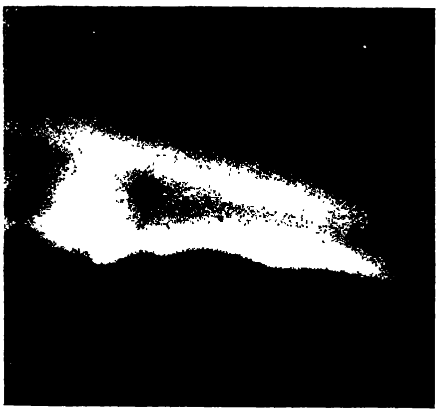
Typical simultaneous photographs of aurora taken at Coral Rapids (C) and Onakawana (O): No. 34 taken Jan. 26, 1931; No. 39 and 40 taken Jan. 27, 1931.



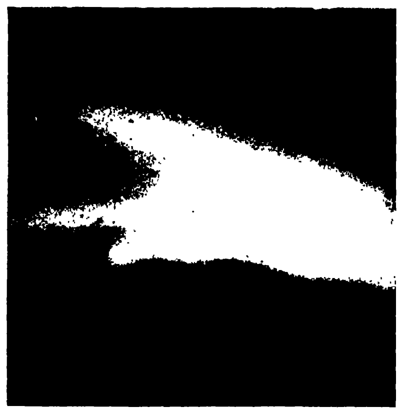
C 41



O 41

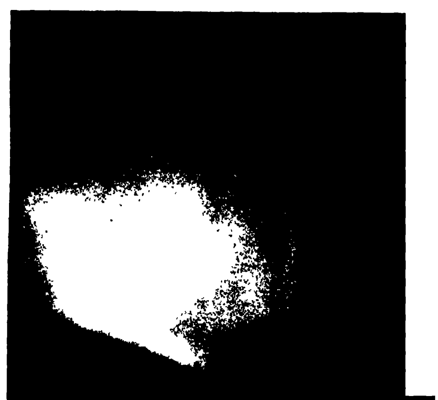


C 45



O 45

C 48



O 48

Typical simultaneous photographs of aurora taken at Coral Rapids (C) and Onakawana (O): No. 41 taken Jan. 27, 1931; No. 45 and 48 taken Feb. 13, 1931.

ON THE SOUND FIELD IN THE NEIGHBORHOOD OF AN OSCILLATING PLANE DISK[1]

BY R. RUEDY[2]

Abstract

With the aid of recent theories (H. Stenzel, N. W. McLachlan, H. Backhaus) the velocity potential and pressure distribution at points in the field of vibrating solid disks of 1 to 20 cm. diameter are calculated for a number of frequencies of practical importance. The graphs drawn from these values apply also to very high frequencies, but smaller disks (1 to 20 mm.). They illustrate the gradual transition from spherical distribution to directed transmission.

The calculation of the sound field in the neighborhood of solid disks or pistons set into an opening of a practically infinite wall and vibrating to and fro with a maximum velocity, v_0, has become of great interest for a number of applications. The methods hitherto used were borrowed from optical theories (4, 5).

At points in the neighborhood of the axis the amplitude distribution may be found by dividing the disk into a number of half-wave zones, and at large distances from the centre the angle with the normal for the first minimum of intensity is equal to $\sin^{-1} 0.61 \frac{\lambda}{c}$. When the diameter of the piston surface is small compared with the wave-length, then the motion is distributed spherically with the disk as centre. But when the wave-length is much smaller than the diameter, zones of maximum and minimum intensity are produced along certain directions around the normal to the centre of the piston, and also along certain more or less circular zones. Close to the vibrating body the wave must be nearly plane.

This theory is only approximate, and whereas in the optical case the number only and the position of the main beams are of most interest, in the sound field the shape of the different beams matters, and effects close to the source have to be studied. A more rigorous treatment has been given recently by H. Backhaus (1).

Taking Lord Rayleigh's equation for the velocity potential, φ, in the case of the circular piston of radius ρ,

$$\varphi = -\frac{1}{2\pi}\iint \frac{\partial\varphi}{\partial n}\,\frac{e^{-ikr}}{r}\,dS,$$

or for a solid body,

$$\varphi = -\frac{1}{2\pi}\frac{\partial\varphi}{\partial n}\iint \frac{e^{-ikr}}{r}\,dS.$$

From the velocity potential the pressure changes at any moment are

$$p = -s\frac{\partial\varphi}{\partial t},$$

[1] *Manuscript received June 18, 193-.*
Contribution from the National Research Laboratories, Ottawa. This paper was read before Section III of the Royal Society of Canada in May, 1931.

[2] *Junior Research Investigator, National Research Laboratories, Ottawa.*

and the velocity along the direction to the centre of the disk

$$v = \frac{\partial \varphi}{\partial r}.$$

Assuming that the integral on which φ depends may be expressed as a sum of spherical harmonics, the new theory gives for the frequency, f, at a point r, θ.

$$\varphi = -v_0 \rho \sqrt{\frac{\pi}{2}} \sqrt{\frac{B_1^2 + B_2^2}{kr}} \sin (2\pi f t + \psi),$$

$$\tan \psi = \frac{B_2}{B_1},$$

with

$$k = \frac{2\pi}{\lambda}$$

$$B_1 = A_0(k\rho)P_0(\cos\theta)J_{\frac{1}{2}}(kr) + A_2(k\rho)P_2(\cos\theta)J_{\frac{5}{2}}(kr) + A_4(k\rho)P_4(\cos\theta)J_{\frac{9}{2}}(kr) + \ldots$$

$$B_2 = A_0(k\rho)P_0(\cos\theta)J_{-\frac{1}{2}}(kr) + A_2(k\rho)P_2(\cos\theta)J_{-\frac{5}{2}}(kr) + A_4(k\rho)P_4(\cos\theta)J_{-\frac{9}{2}}(kr) + \ldots$$

$$A_{2n}(k\rho) = (-1)^n(4n+1)\left[\frac{1}{k\rho} - \frac{1}{2_n}\sum(-1)^{n-m}\frac{1.3.5\ldots(2n+2m-1)}{m!(n-m)!}\frac{\sqrt{\frac{\pi}{2}}\,J_{m-\frac{1}{2}}(k\rho)}{(k\rho)^{m+\frac{1}{2}}}\right].$$

for instance,

$$\frac{8}{9}k\rho\,A_4(k\rho) = 8 - \cos k\rho\left(3 - \frac{105}{(k\rho)^2}\right) + \frac{\sin k\rho}{k\rho}\left(30 - \frac{105}{(k\rho)^2}\right)$$

$$-\frac{16}{13}k\rho\,A_6(k\rho) = 16 + \cos k\rho\left(5 - \frac{945}{(k\rho)^2} + \frac{10395}{(k\rho)^4}\right) - \frac{\sin k\rho}{k\rho}\left(105 - \frac{4410}{(k\rho)^2} + \frac{10395}{(k\rho)^4}\right)$$

$$\frac{16}{17}k\rho\,A_8(k\rho) = 16 - \cos k\rho\left(\frac{35}{8} - \frac{10395}{4(k\rho)^2} + \frac{1216215}{8(k\rho)^4} - \frac{101351251}{8(k\rho)^6}\right)$$

$$+ \frac{\sin k\rho}{k\rho}\left(\frac{315}{2} - \frac{100485}{4(k\rho)^2} + \frac{4590590}{8(k\rho)^4} - \frac{10135125}{8(k\rho)^6}\right).$$

P = spherical harmonics; J = Bessel functions.

This solution is valid for points of the sound field facing the piston lying outside a hemispherical cap of radius equal to that of the disk; it also applies to all the points lying upon the normal to the centre of the disk. In comparison with the approximate theory the rigorous treatment gives a smaller number of directions and points in which there is no pressure change, less narrow and stronger sidebeams and therefore more overlapping. This feature has been emphasized in experiments on ultrasonics (2) and has also been verified in the case of low frequency waves.

In an attempt to extend the solution to points lying inside the hemispherical cap, at least for ultrasonic frequencies, calculations were made for the frequency, 54100 cycles per sec., or a wave-length of 0.63 cm. as emitted by pistons of diameters $2\rho = 0.2$, 0.63, 1.26 and 1.88 cm. When multiplied by ten the results are also valid for wave-lengths ten times longer, provided the piston diameters and distances are enlarged in the same ratio so that the values of $\frac{2\pi\rho}{\lambda}$ and $\frac{2\pi r}{\lambda}$ remain unchanged, that is, for a frequency of 5410 periods per sec. and piston diameters of 2, 6.3, 12.6 and 18.8 cm., or loud-speaker dimensions.

To avoid too complicated figures, only the curves $\sqrt{\frac{B_1^2+B_2^2}{kr}}$ for angles θ equal to zero, thirty or sixty degrees from the normal, have been plotted. The curves show the gradual transition from diffuse spherical to directed radiation affected by interference, and as their accuracy may be tested experimentally, they are thought to be of general interest as interference effects on a convenient scale.

As for sound waves the pressure amplitude of the air is proportional to the velocity potential φ, the curves constructed from the computed values may be considered as giving the maximum pressure amplitudes occurring at any time at points before the disk. In order to obtain the instantaneous distribution

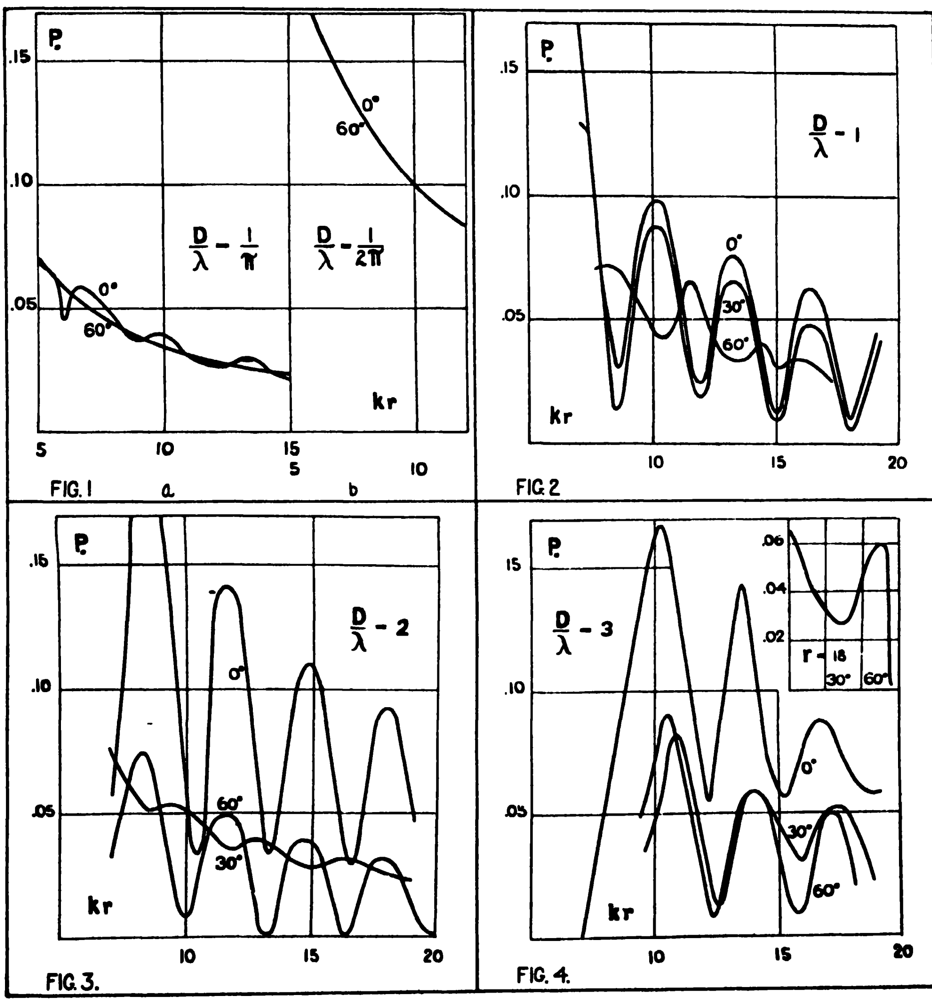


FIG. 1–4. *Pressure amplitudes in the sound field of a vibrating solid disk of diameter D.*

of pressure the phase angle, ψ, must be taken into account; the slope of the tangents to the curves gives then the velocity of vibration in the direction to the centre of the disk.

With regard to the absolute values of the pressure changes at any point (c = velocity of sound),

$$p = -2\pi s v_0 \rho f \sqrt{\frac{\pi}{2}} \sqrt{\frac{B_1^2 + B_2^2}{kr}} \cos(2\pi f t + \psi),$$

or

$$p = -1.253\, c\, v_0\, (k\rho) \sqrt{\frac{B_1^2 + B_2^2}{kr}} \cos(2\pi f t + \psi).$$

When plotting the values of the square root only as has been done in drawing the graphs, the absolute value of the amplitude at a point may be found by multiplying by $1.253\, scv_0\, k\rho$ (where s is the density of the air, c the velocity of sound), except in the case of Fig. 1*b* where the factor is $0.132\, scv_0$; actually there are phase differences between the different values. The points have been computed for every one-half centimetre distance, putting $k = 1$.

In the first case, diameter equal to 1 mm., the curves for all the angles from 0° to 90° from the normal practically coincide and show an almost perfectly regular decrease for increasing distances. The sound waves are essentially spherical waves.

In the case where the diameter is equal to 2 mm., marked interference effects make themselves felt; at certain distances the amplitudes remain small, but there is little difference as to maximum amplitudes when different angles are considered.

In the second figure, diameter comparable to wave-length, $k\rho = \pi$, the interference effects cut deeper; at certain distances the amplitudes tend to zero, thus giving the appearance of node formation. At the same time, the distribution valid for zero degree differs completely from that at ninety degrees and the tendency to form side beams becomes apparent.

For $k\rho = 3\pi$(in Fig. 4) one point upon the normal to the disk may be found in which the pressure amplitude is zero. Several attempts to verify this feature have failed. The number of points on the normal with no pressure change would increase when $k\rho$ increases. It is possible that frictional forces tend to produce a flow of the air and smooth out the differences between the points of greatest and least changes.

The up-and-down fluctuations of the pressure amplitude along the different directions grow gradually smaller as larger values of r are examined, and finally at distances at which the lines connecting a point with all the different surface elements of the disk may be considered as parallel lines, Airy's solution holds(6).

$$\varphi = -\frac{\rho^2 v_0}{r} \frac{J_1(k\rho \sin\theta)}{k\rho \sin\theta} \cos(2\pi f t - kr),$$

$$p = -\frac{s\partial\varphi}{\partial t} = s\, c\, v_0\, k\rho \frac{J_1(k\rho \sin\theta)}{kr \sin\theta} \sin(2\pi f t - kr).$$

The graphs may also be used in another way. When the curves for $k\rho = 3\pi$ are taken as applying to a solid vibrating disk of diameter 6π cm., they represent the pressure distribution for a wave of 5410 cycles (or a wave-length of 6.28 cm. in air), that is, for a very high note. The graphs for the case $k\rho = 2\pi$ may then be considered as applying to the same disk but to a three times longer wave-length and at three times longer distances; those for $k\rho = \pi$ to six times enlarged dimensions (frequency 900 cycles), and for $k\rho = 1$ the dimensions would be measured in dm. instead of in cm. For this latter case and all the lower frequencies (<500 cycles), the distribution is nearly hemispherical, and the formula contains only terms with A_0 so that (Fig. 1-*b*.)

$$p = -s\,c\,v_0\,\frac{1-\cos k\rho}{kr}\cos(2\pi ft+\psi),$$

because

$$\frac{2}{\pi y} = J^2_{\frac{1}{2}}(y) + J^2_{-\frac{1}{2}}(y).$$

The different curves thus show the main features of the sound field for the frequencies of practical importance and the deviation from uniform distribution when high frequencies are considered. It is important that as far as measurements and practical uses are concerned the ideal disk oscillating at high frequency may be closely imitated if instead of cutting thin quartz slabs as circular disks, the different diameters are made proportional to the square root of the modulus of elasticity in that direction (7). At lower frequencies the disks may be driven electromagnetically. As the conditions in the sound field may be determined by merely measuring the amplitude of vibration of the disk, the disks are suitable for standard and precision sound sources. The theory applies to more complicated structures provided they can be resolved into or replaced by a number of circular disks.

It is desirable to extend the graphs to larger values of $k\rho$; the amount of computation increases rapidly however with $k\rho$ and suitable tables for the higher order Bessel functions will have to be prepared.

Acknowledgment

This work was proposed by Dr. R. W. Boyle and thanks are due to him for suggestions received.

References

1. Backhaus, H. Ann. Physik, 5: 1-35. 1930.
2. Boyle, R. W., Lehmann, T. F. and Reid, C. D. Trans. Roy. Soc. Can. 19 III: 167-196. 1925.
3. Lindsay, R. B. Phys. Rev. 32: 515-519. 1928.
4. McLachlan, N. W. Proc. Roy. Soc. Lond. 122A: 604-609. 1929.
5. Stenzel, H. Elek. Nachr.-Tech. 4: 239-251. 1927.
6. Stenzel, H. Ann. Physik, 7: 947-982. 1930.
7. Straubel, H. Physik. Z. 32: 222. 1931.

STUDIES ON LIGNIN AND RELATED COMPOUNDS
IX. ETHERS OF GLYCOL-LIGNIN[1]

By Harold Hibbert[2] and Léo Marion[3]

Abstract

Glycol-lignin prepared from spruce wood has been found to form a sodium salt which reacts with chloro-derivatives, giving rise to the corresponding glycol-lignin ethers. These compounds are insoluble in aqueous sodium hydroxide but still contain free hydroxyl groups which can be methylated without the ethereal linkage being broken. Hence glycol-lignin contains several free hydroxyl groups, one or more of which possess acidic properties. Both the methoxymethyl ether and the 2:4-dinitrophenyl ether of glycol-lignin have been prepared. The evidence would seem to indicate the presence of both phenolic and aliphatic hydroxyl groups.

Introduction

Recently an account was given of some investigations (5, 6) which supplied evidence that could be interpreted as an indication of the presence in glycol-lignin of a phenolic nucleus. The work described in the present paper and carried out simultaneously with the latter, was designed to emphasize any difference in the nature of the hydroxyl groups present in glycol-lignin.

Glycol-lignin, isolated from spruce wood by means of glycol containing a trace of hydrogen chloride, contains free hydroxyl groups (4). It appeared possible, therefore, to prepare a sodium salt which might be made to react with various chloro-derivatives to yield the corresponding ethers. The present work is concerned with the preparation of such ethers of glycol-lignin and a study of their properties.

Hoering and Baum (7) prepared methoxymethyl phenyl ether by allowing sodium phenolate to react with chlorodimethyl ether. By the application of the same method, Pauly and Wäscher (9) and, later, Pauly and Feuerstein (8) succeeded in obtaining coniferyl aldehyde from vanillin. In a somewhat similar manner, Freudenberg and Hess (1) prepared a *p*-toluene sulphonic ester of Willstätter lignin which they were able to isolate and decompose by treatment with hydroxylamine. Their purpose was to determine the nature of the hydroxyl group with which the sulphonic radical was combined. Assuming a molecular weight for lignin of the order of 800, they concluded that it contained four aliphatic, or hydroaromatic, hydroxyl groups, and one phenolic group. Using a lignin prepared by a different method, however, Freudenberg, Zocher and Dürr (2) later concluded that phenolic groups are absent in the lignin complex.

As a first step towards the synthesis of ethers of glycol-lignin, the sodium salt of the latter was prepared from its alcoholic solution by the addition of sodium

[1] *Manuscript received June 24, 1931.*
Contribution from the Department of Industrial and Cellulose Chemistry, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada and the Canadian Pulp and Paper Association.

[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*

[3] *Assistant Research Chemist, National Research Laboratories, Ottawa, and holder, at the time, of a studentship under the National Research Council.*

ethylate. The salt is a very hygroscopic, light-colored, amorphous powder which is oxidized to a dark-brown mass when left exposed to the air, a property characteristic of the sodium salts of polyhydroxy phenols.

The sodium salt of glycol-lignin, when suspended in toluene and allowed to react under suitable conditions with 1:2:4 chlorodinitrobenzene, yielded a lemon-yellow compound containing 2.74% nitrogen and having a methoxyl content of 13.60%. Its molecular weight, determined cryoscopically in glacial acetic acid, had an average value of 499. The ether is insoluble in dilute sodium hydroxide, but undergoes methylation by the agency of dimethyl sulphate and potassium hydroxide, the methoxyl content being increased to 17.0% (average of four determinations). If, however, the temperature during methylation be allowed to rise to 90-100° C., and the reaction mixture to become alkaline, the ether group is removed and the insoluble lignin derivative has, after purification, a methoxyl content of 22.63%. Fully methylated glycol-lignin has a methoxyl value of 26.26%. If, therefore, it be assumed that the difference between the two compounds consists in one acidic hydroxyl group being free in the one case and methylated in the other, the molecular weight of fully methylated lignin is found by calculation to be 766. This figure, however, can only be considered as representing the order of the size of the molecule, owing to the possibility of the product left after removal of the dinitrophenyl group having undergone partial methylation. In fact, the molecular weight of fully methylated glycol-lignin can be calculated from the methoxyl values of fully methylated glycol-lignin (26.26%) and of the fully methylated ether (17%) to be 615.

The results show, however, that glycol-lignin contains at least two types of hydroxyl groups and that those responsible for the solubility of glycol-lignin in dilute sodium hydroxide can combine with the sodium present in sodium ethoxide to form a well-defined salt. Fuchs and Horn (3) found that lignin isolated from spruce wood contains two kinds of hydroxyl groups, some undergoing methylation through the agency of diazomethane while the others are inert towards this reagent; this is in harmony with the above conclusion.

Chlorodimethyl ether also reacts with the sodium salt of glycol-lignin, yielding the methoxymethyl ether of glycol-lignin. The methoxyl content of the compound has an average value of 18.9%. As in the case of the dinitrophenyl ether this compound is insoluble in dilute sodium hydroxide but can be methylated.

Experimental

Preparation of the Sodium Salt of Glycol-lignin

About 2 gm. of moist glycol-lignin* was dissolved in 100 cc. of absolute alcohol and the solution cooled to −10° C. A solution of sodium ethylate (1 gm. metallic sodium in 20 cc. absolute alcohol), previously cooled to the same temperature, was gradually added, with occasional shaking, to the cooled alcoholic solution. The sodium salt of glycol-lignin immediately separated as

**Glycol-lignin was prepared and purified as described in a previous communication (4) and kept moist in a well-stoppered brown flask until needed.*

a light-colored precipitate which was washed by decantation with cold absolute alcohol, filtered as rapidly as possible, and washed on the filter, first with absolute alcohol and then with anhydrous ether. It was spread on a watch glass and dried over phosphorus pentoxide in an evacuated desiccator. The product was left as a hygroscopic powder which gradually turned dark brown when left exposed to the air. It was insoluble in ether and alcohol (absolute); soluble in water; also soluble in pyridine with decomposition. The substance precipitated from the pyridine solution by the addition of ether or absolute alcohol is no longer soluble in water.

Dinitrophenyl Ether of Glycol-lignin

The dry sodium salt of glycol-lignin (2.5 gm.) was suspended in 50 cc. of toluene in a flask, equipped with a mechanical stirrer, and heated in a bath kept at 70° C. To this was then added a solution of 2 gm. of 1:2:4 chlorodinitrobenzene in 50 cc. of toluene, and the mixture stirred at 70° C. The dinitrophenyl ether gradually separated as a lemon-yellow precipitate, together with sodium chloride. After five hours the mixture was cooled and water added to dissolve the sodium chloride and any unchanged sodium salt of lignin. The solid ether was then filtered, washed with water, and dried under reduced pressure. It was purified by dissolving in glacial acetic acid (about 40 cc.) and reprecipitated by pouring the solution into 500 cc. water. The ether thus obtained, after filtering, washing and drying (yield 2.6 gm.) was a light lemon-yellow, amorphous powder soluble in glacial acetic acid, very slightly soluble in dilute alcohol and insoluble in toluene, acetone, chloroform and ethyl acetate. Analysis: C, 57.82, 57.70%; H, 5.93, 5.73%; N, 2.71, 2.78%; (OCH_3), 13.84, 13.60, 13.26%. Mol. wt. in glacial acetic acid: sample, 0.0498, 0.0604 gm., Δ,0.193, 0.231° C., mol. wt., 496, 502.

Methylation of Glycol-lignin Dinitrophenyl Ether

The dinitrophenyl ether (0.5 gm.) was suspended in 5 cc. of 1% sodium hydroxide and methylated by the addition of 5 cc. of dimethyl sulphate and 5 cc. of 50% potassium hydroxide at 43-45° C. Both reagents were added at such a rate as to maintain the mixture as nearly neutral as possible throughout the addition. The reaction product was filtered, washed and then subjected to a second similar methylation. Following this second treatment, the product was filtered, washed with water and dried under reduced pressure over phosphorus pentoxide. It was obtained as a light-brown powder, insoluble in sodium hydroxide, alcohol or glacial acetic acid. Analysis: CH_3O, 17.15, 16.42, 16.93, 17.55%. If during methylation, the temperature was allowed to rise to 90-100° C. and the reaction mixture to become alkaline, the dinitrophenyl ether group was removed, leaving a partially methylated lignin derivative. Analysis: CH_3O, 22.78, 22.48%.

Methoxymethyl Ether of Glycol-lignin

This ether was obtained by the interaction of chlorodimethyl ether with the sodium salt of glycol-lignin. Two grams of the salt was suspended in pure, dry toluene, and the mixture, after cooling in ice, stirred with 15 cc. of chloro-

dimethyl ether for one hour. The temperature of the reaction mixture was then allowed to rise to room temperature and the product poured into a large volume of water to dissolve any of the unchanged sodium salt of lignin and the sodium chloride formed. The methoxymethyl ether, after filtering, washing and drying under reduced pressure over phosphorus pentoxide, was a light-brown, amorphous powder, insoluble in toluene, ether and dilute caustic soda. Analysis: C, 61.79, 61.42%; H, 6.32, 6.06%; CH_3O, 19.08, 19.61%.

References

1. Freudenberg, K. and Hess, H. Ann. 448: 121-133. 1926.
2. Freudenberg, K., Zocher, H. and Dürr, W. Ber. 62: 1814-1823. 1929.
3. Fuchs, W. and Horn, O. Ber. 62: 1691-1693. 1929.
4. Hibbert, H. and Marion, L. Can. J. Research, 2: 364-375. 1930.
5. Hibbert, H. and Sankey, C. A. Can. J. Research, 4:110-118. 1931.
6. Hibbert, H. and Taylor, K. A. Can. J. Research, 4: 240-253. 1931.
7. Hoering, P. and Baum, F. Centr. 1: 1680. 1909.
8. Pauly, H. and Feuerstein, K. Ber. 62: 297-311. 1929.
9. Pauly, H. and Wäscher, K. Ber. 56: 603-610. 1923.

THE PREPARATION AND PHYSICAL PROPERTIES OF ETHYL AND METHYL ACETYLENE[1]

By F. R. Morehouse[2] and O. Maass[3]

Abstract

The method for preparing an alkyl acetylene by the action of an alkyl halide on sodium dissolved in liquid ammonia was modified in certain details. Ethyl and methyl acetylene were prepared and purified and their melting and boiling points determined. Vapor pressures, densities and surface tensions were measured over a considerable temperature range. These properties together with constants calculated from the data are compared with similar data for paraffins and olefines.

A systematic study of the physical properties of aliphatic hydrocarbons has been undertaken in this laboratory. Those containing two and three carbon atoms were investigated by Wright and Maass (4) and the properties of the four-carbon paraffins and olefines were determined by Coffin and Maass (1). In this paper the first of the four-carbon acetylenes is the subject of investigation.

Certain details in the method of preparation which lead to better yields are described. The method of purification was similar to that used by Coffin and Maass (1). Since this is a marked improvement over that used by Wright and Maass (4), where small amounts of material are concerned, the preparation of methyl acetylene also was undertaken to see if, when purified in this way, the results of Wright and Maass would be confirmed or not.

Preparation

The acetylene compounds were prepared by the action of the alkyl halides on sodium acetylide in a manner analogous to that described by Lebeau and Picon (2). At first the type of apparatus described by Russell and Maass (3) was employed and special care was taken to dry the ammonia in sodium hydroxide towers and to add the alkyl halide after the excess acetylene had been removed. It was found that a very poor yield was obtained even though, after addition of alkyl halide, the reaction mixture had been allowed to stand for some time. This was attributed to the low temperature (−78° C.) of the carbon dioxide-ether bath in which it was kept. The maximum temperature at which the reaction mixture can be maintained is of course governed by the boiling point of the ammonia.

The apparatus was therefore modified by the addition of a reflux condenser (Fig. 2) whereby the alkyl halide could be added while the mixture was being refluxed; *i.e.*, at the highest possible temperature. Fig. 1 represents the apparatus used. It was constructed so as to be adapted to the preparation of other acetylenic compounds and to be a permanent set-up.

[1] *Manuscript received March 16, 1931.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada.

[2] *Holder, at the time, of a studentship under the National Research Council of Canada.*
[3] *Professor of Physical Chemistry, McGill University.*

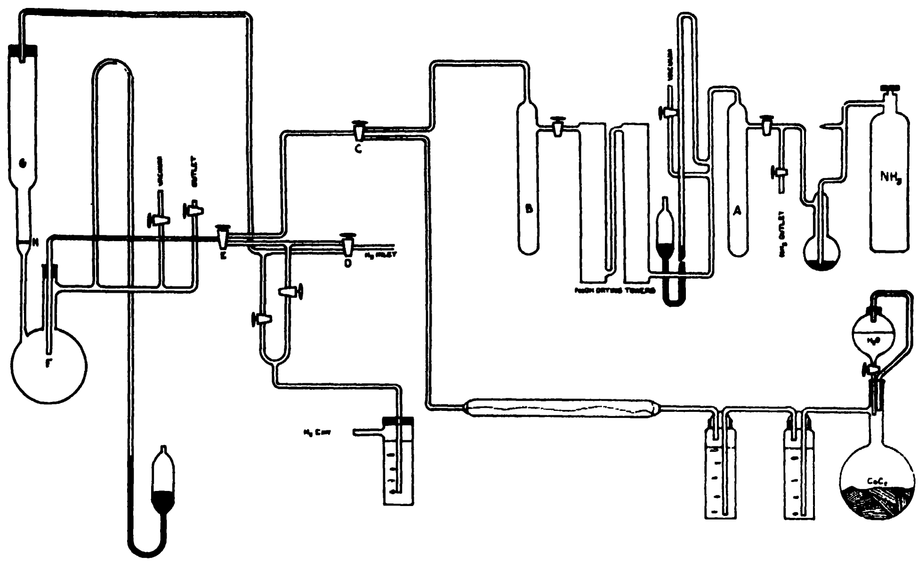


FIG. 1. *Apparatus used in preparation of alkyl acetylenes.*

The ammonia is passed through sodium hydroxide drying towers and then condensed in the reaction flask, *F*, where it dissolves the sodium, previously melted in an atmosphere of hydrogen in the tube *G*. It is then strained, while molten, through the perforated porous plate, *H*, in the reaction flask. When the sodium has been added tube *G* is sealed off. Pure acetylene is then passed through the solution of sodium in liquid ammonia until the blue color disappears after which the reflux condenser, Fig. 2, is attached to the reaction flask and the contents allowed to reflux for some time to remove any dissolved acetylene. The reflux condenser consists of a tube jacketed with carbon dioxide-ether mixture. A dropping funnel is attached for the addition of the alkyl halide. After it has been added the contents of the flask are allowed to boil off through a scrubbing apparatus (Fig. 3) in which the flask, *C*, contains a small amount of water kept acid by the gradual addition of sulphuric acid from a dropping funnel, a piece of litmus paper in the solution acting as indicator. In this way complete removal of ammonia was assured. Yields obtained by the use of this type of separator were far greater than when the larger part of the ammonia was first removed by passing the gases through a large quantity of water; 70% yields were obtained.

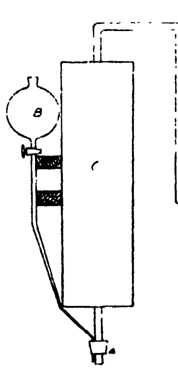


FIG. 2. *Reflux condenser.*

The alkyl acetylene compound was then collected and purified by the system of fractional distillation used by Coffin and Maass. Vapor density determinations were made at different pressures on the ethyl acetylene, giving a result which corresponded to the theoretical molecular weight. Pure methyl

acetylene was obtained by distilling until a constant vapor pressure for different fractions was obtained. The vapor pressures of the final two fractions were 54.5 and 54.3 cm. Hg. at −30° C.

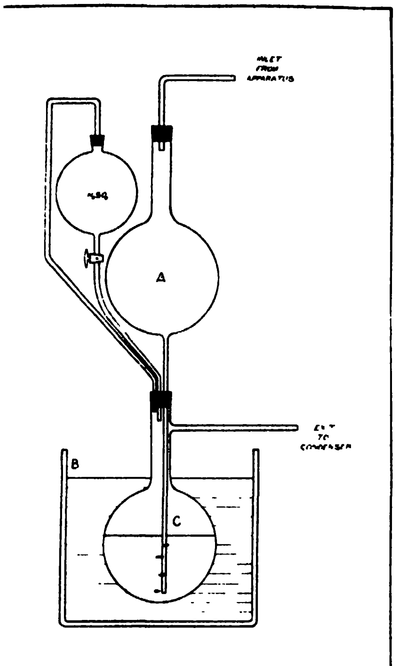


FIG. 3. *Scrubbing apparatus.*

Ethyl and methyl acetylene were prepared twice with slight changes in the method of procedure. In the first preparation of ethyl acetylene it was never at any time in contact with an acid separator which might have polymerized it. In the second preparation, where better yields were obtained by the use of the acid separator described above, the alkyl acetylene was in contact with acid both in the separator and also during passage over phosphorus pentoxide for removal of small traces of water. Most of the water was first removed by chilling and then filtering off the ethyl acetylene. Although propylene polymerizes (4) on being passed over phosphorus pentoxide, ethyl acetylene does not appear to do so since the vapor pressures and boiling points were found to be the same whether it did or did not come into contact with an acid medium.

The useful information was therefore obtained that no secondary products are formed by passing the alkyl acetylene through dilute sulphuric acid or (after removal of most of the water) by passing it over phosphorus pentoxide. Consequently both ammonia and water can be completely removed before fractionation. The data for ethyl acetylene given below are for two samples prepared separately from the start. The results obtained with preparation I are from the sample which had never been in contact with an acid solution or phosphorus pentoxide, while those for preparation II are from the sample which was passed through the acid solution and over phosphorus pentoxide. Both sets of data are seen to agree.

Determination of the Melting Point

Melting points were determined by freezing the hydrocarbons in small bulbs which were immersed in a bath of gasoline contained in a transparent Dewar flask. A Pyrex test tube was held in the bath and liquid air was put in it until the desired temperatures were reached. Stirring of the gasoline was effected by a current of dry air.

Temperatures were measured by a standardized thermometer completely immersed in the liquid. The melting points of ethyl and methyl acetylene were found to be −122.5° and −101.5° C. respectively.

Boiling Point

Ethyl Acetylene

The boiling point of ethyl acetylene has been found by different investigators and considerable disagreement occurs in the literature. The boiling point was found from the vapor pressure curve taken over a suitable temperature range. Temperatures were maintained by an air-stirred ether bath cooled with solid carbon dioxide and measured with a standard thermometer. The following tables give the vapor pressures for preparation I and preparation II from which the boiling point in both sets of data is found to be +8° C.

TABLE I

VAPOR PRESSURES OF ETHYL ACETYLENE

Preparation I				Preparation II			
Temp., ° C.	Vapor pressure, cm. Hg.	Temp., ° C.	Vapor pressure, cm. Hg.	Temp., ° C.	Vapor pressure, cm. Hg.	Temp., ° C.	Vapor pressure, cm. Hg.
−38.8	6.80	−5.7	43.10	−32.4	11.46	−7.0	40.85
−30.5	12.38	−1.3	50.78	−24.1	17.93	−4.5	46.03
−21.3	20.67	+3.5	61.96	−21.3	19.93	+8.0	74.42
−16.9	26.18	+9.1	77.66	−17.4	24.81	+9.5	78.80
−10.8	34.60			−11.4	33.57		

Methyl Acetylene

The boiling point of methyl acetylene was found in the same way. The boiling point in preparation I (−23.3° C.) was found to be the same as that in preparation II within 0.1° C. This value for the boiling point shows fair agreement with that (−23.0° C.) obtained by R. N. Meinert and C. D. Hurd (5).

TABLE II

VAPOR PRESSURES OF METHYL ACETYLENE

Preparation I				Preparation II			
Temp., ° C.	Vapor pressure, cm. Hg.	Temp., ° C.	Vapor pressure, cm. Hg.	Temp., ° C.	Vapor pressure, cm. Hg.	Temp., ° C.	Vapor pressure, cm. Hg.
−54.1	15.88	−33.4	48.74	−50.1	20.03	−25.2	71.15
−49.6	20.46	−28.5	60.80	−39.6	35.18	−22.6	80.01
−44.0	27.64	−25.5	67.80	−30.2	55.90	−21.1	83.80
−36.5	42.17	−22.5	79.70				

Density

Density determinations were made by the dilatometer method described by Wright and Maass (4). The dilatometer was glass-sealed to the apparatus and filled by distillation. In the tables below are given the densities of ethyl and methyl acetylene for preparations I and II.

TABLE III

DENSITIES OF ETHYL ACETYLENE

Preparation I		Preparation II	
Temp., ° C.	Density	Temp., ° C.	Density
−28.0	0.7095	−31.3	0.7119
−20.0	0.6997	−23.0	0.7028
−15.0	0.6949	−11.0	0.6899
−10.5	0.6900	− 0.2	0.6784
− 5.0	0.6836	+ 8.9	0.6685
− 0.3	0.6784		
+ 5.6	0.6706		

TABLE IV

DENSITIES OF METHYL ACETYLENE

Preparation I				Preparation II			
Temp.,°C.	Density	Temp.,°C.	Density	Temp.,°C.	Density	Temp.,°C.	Density
−14.4	0.6617	−36.8	0.6873	− 0.5	0.6447	−33.0	0.6814
−17.5	0.6652	−42.4	0 6936	−12.7	0.6582	−40.9	0.6911
−22.7	0.6710	−47.4	0.6992	−24.2	0.6711	−51.9	0.7039
−26.5	0.6759	−53.1	0.7065				
−32.5	0.6827	−57.0	0.7109				

Surface Tension

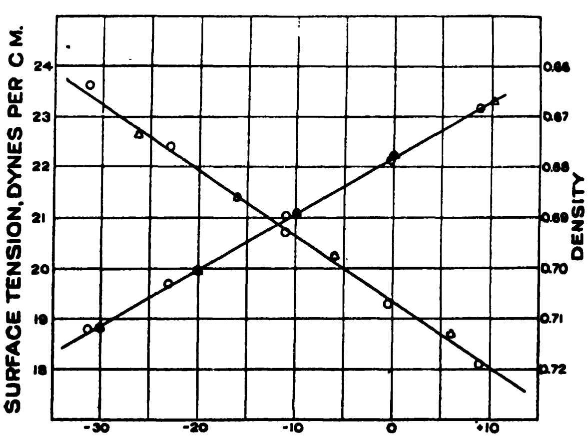


FIG. 4. *Surface tension and density of ethyl acetylene.*

The surface tension of ethyl acetylene was found by the use of a capillarimeter which was glass-sealed to the purifying apparatus and filled by distillation. A description of this method and the accuracy obtained is to be found in the paper by Wright and Maass (4) referred to above. The following table gives the values obtained for surface tension. The curves for surface tension derived from both sets of data for ethyl acetylene are shown in Fig. 4.

TABLE V

SURFACE TENSION OF METHYL AND ETHYL ACETYLENE

Methyl acetylene*		Ethyl acetylene			
Preparation II		Preparation I		Preparation II	
Temp., °C.	Surface tension	Temp., °C.	Surface tension	Temp., °C.	Surface tension
−11.5	18.91	−26.3	22.65	−31.3	23.62
−21.0	20.37	−16.0	21.40	−23.0	22.44
−31.6	22.00	− 6.3	20.25	−11.0	20.77
−38.4	23.08	+ 6.5	18.70	− 0.2	19.32
				+ 8.9	18.13

**The surface tension of methyl acetylene was found only under those conditions pertaining to preparation II.*

Discussion

Table VI is a compilation of the data obtained for the hydrocarbons of the acetylene series. A table containing similar data for the paraffins and olefines can be found in a paper by Coffin and Maass (1).

TABLE VI

PHYSICAL PROPERTIES OF ACETYLENES

Property	Acetylene	Allylene	Ethyl acetylene
Melting point, °C.	−81	−101.5	−122.5
Boiling point, °C.	−83.6	−23.3	+8.6
Molecular latent heat of evaporation, cal.	4273	5562	5983
Surface tension at b.p. dynes/cm.	19.6	20.7	18.3
Total surface energy	56.0	59.42	53.5
Ramsay and Shield's constant	2.09	1.99	1.96
Trouton's constant	—	22.3	21.3

Property	Acetylene	Allylene	Ethyl acetylene
Critical temperature, °C.	39.7	121.6	190.5
Molecular volume at b.p., °C.	41.9	59.7	80.8
Parachor:			
Observed	88.1	127.5	167.2
Calculated	90.4	129.4	168.6
Difference as % of observed value	2.6	1.5	0.8

The new data obtained from allylene do not differ markedly from that obtained by Maass and Wright (4) except in so far as the boiling point is concerned. This was found to be considerably higher. The amount of material used by Maass and Wright was considerably less, and it is possible that in the determination of the vapor pressures all the air above the liquid had not been removed. In any case, because of the larger amount of material available for purification, the data for allylene given above may be considered more accurate.

The physical constants obtained for ethyl acetylene fall in line with the analogous constants obtained for the olefines and paraffins on one hand and the two lower acetylenes on the other. The values obtained for Trouton's constant and for Ramsay and Shield's constant show that ethyl acetylene may

be regarded as unassociated liquid just as all the other hydrocarbons which have been examined. As a member of the acetylenes, ethyl acetylene shows a greater force of molecular attraction than the olefines and paraffins. The latent heat of evaporation, the total surface energy, the boiling point and the critical temperature are all considerably higher than the similar constants for the butanes and butylenes. The melting point is higher than for any other "four-carbon" hydrocarbon, and that it is the lowest as compared to the two lower acetylenes is to be expected. There seems to be no question but that the acetylenes have what some like to call a larger residual valency than the corresponding olefines and paraffins.

The molecular volume at the boiling point of acetylene, allylene and ethyl acetylene can only be brought into line with Kopp's values and not those of Le Bas; ethyl acetylene showing the increase of 3.8 units for triple bond on saturation which Kopp found. The two lower members of the acetylene series show the increase above this value which is to be expected from their position in the acetylene series.

The values for the observed parachor are given in Table VI for acetylene, allylene and ethyl acetylene, and there is agreement between these values and those calculated on the basis of Sugden's values. That ethyl acetylene shows the best agreement of the three is to be expected, since it is further up in the series where greater uniformity is to be expected.

References

1. Coffin, C. C. and Maass, O. J. Am. Chem. Soc. 50: 1427-1437. 1928.
2. Lebeau, P. and Picon, M. Compt. rend. 156: 1077-1079. 1913.
3. Maass, O. and Russell, J. J. Am. Chem. Soc. 43: 1227-1230. 1921.
4. Maass, O. and Wright, C. H. J. Am. Chem. Soc. 43: 1098-1111. 1921.
5. Meinert, R. N. and Hurd, C. D. J. Am. Chem. Soc. 52: 4540-4549. 1930.

FACTORS WHICH INFLUENCE SPONTANEOUS SELF-FERTILIZATION IN SWEET CLOVER (MELILOTUS)[1]

By L. E. Kirk[2] and T. M. Stevenson[3]

Abstract

Seven factors have been described which seem to influence the tendency of sweet clover plants to produce seed by spontaneous self-fertilization. These are: length of stamens; stage of flower development when pollen is liberated from the pollen sacs; distribution of free pollen within the flower; size of cavity in the upper part of the keel; amount of pollen; condition of the pollen; and receptivity of the stigma.

Spontaneous self-fertilization is the normal condition in certain plants of white blossom sweet clover, *i.e.*, *M. alba*. This was not found to occur in any variety of yellow blossom sweet clover with the exception of "Redfield Yellow". True breeding lines of *M. alba* have been isolated consisting of naturally self-fertilized plants and other lines also which will not produce seed unless the flowers are manipulated. *M. alba* consists of a mixture of normally self-fertilized plants and plants that are random pollinated. This is especially true of certain varieties such as "Arctic". In some plants of *M. alba* an abundance of germinating pollen grains were found adhering to the stigmas before the flowers opened, thus precluding the possibility of natural crossing. Practically 100% of the flowers on such a plant produced selfed seed. The character of spontaneous self-fertilization exhibited clear cut segregation in a selfed line of *M. alba*. The importance of this character in relation to breeding improved varieties and pure seed production is emphasized.

The efficiency of the "suction" method of emasculating sweet clover flowers depends on the peculiarities of the latter. This method is quite reliable with many plants but very unsatisfactory with others, depending upon the particular type of flower structure and behavior.

Previous studies indicated that plants of *Melilotus alba* will set seed freely without artificial manipulation of the blossoms and when the plants are screened to prevent pollination by insects, whereas *Melilotus officinalis* sets almost no seed when the blossoms are undisturbed by artificial means. We now believe that these findings require considerable modification, since our studies have shown that both the white and yellow blossom species of *Melilotus* contain strains which are naturally self-fertilized. We have also learned something of the mechanism which is responsible for differences in seed setting under controlled conditions of pollination. Results of these studies are given in the following pages, together with a discussion of their significance in relation to methods of breeding better varieties of sweet clover.

Literature Review

Kirchner (4) enclosed flowers of *M. alba* in gauze bags and concluded from a limited study that spontaneous self-fertilization was of general occurrence. Coe and Martin (1) conducted an extensive series of pollination studies with *M. alba*. In their experiments the flowers were enclosed in tarlatan, a material which has a weave twice as close as ordinary gauze. These investigators

[1] *Manuscript received June 12, 1931.*
Contribution from the Field Husbandry Department, University of Saskatchewan, Saskatoon, Sask.

[2] *Professor of Field Husbandry, University of Saskatchewan.*

[3] *Research Assistant, University of Saskatchewan.*

obtained less than one pod per raceme from protected plants, and concluded that "spontaneous self-pollination does not occur regularly as stated by Kirchner".

Kirk (5) studied the question of spontaneous self-fertilization in both *M. alba* and *M. officinalis*. The plants were completely enclosed in cages three feet square and five feet high, made of medium weight cotton with a weave of 52 strands to the inch. *M. alba* produced an average of 34.9 pods per raceme when enclosed in cotton cages as compared with 66.4 pods per raceme for open-pollinated plants. *M. officinalis*, on the other hand, gave only 2.6 pods per raceme as compared with 63.9 pods for open-pollinated plants. The difference between the two species was very pronounced, and the seed setting of protected plants was consistently in favor of *M. alba*. It was concluded from this study that *M. alba* will set seed freely without artificial manipulation or the visitation of insects, but that *M. officinalis* will produce very few pods when insects are excluded.

More recently the results of pollination studies with sweet clover have been published by Dann (2) and Ufer (8). Dann used one paper bag to enclose several racemes on each of 10 plants of *M. alba*. In two bags no pods were obtained, the other plants giving 4.0, 8.5, 9.0, 9.2, 18.2, 21.1, 59.0 and 60.0%. He assumes in the last two cases that small insects may have gained access to the flowers, and concludes that *M. alba* is capable of natural self-fertilization to a slight degree only, but entirely self-fertile on artificial selfing. *M. officinalis*, on the other hand, produced no pods in any of the bags and was therefore judged to be incapable of seed setting without artificial manipulation.

Ufer studied the question of seed setting in *Melilotus* by protecting the plants from insect visitation with pergamin bags of two sizes, both with and without ventilation. Seven biennial and five annual species and varieties were included in his experiments. Owing to unfavorable weather conditions and the possible effects of unnatural conditions within the bags, the author did not consider that a true picture of the actual facts had been obtained. At the same time he was reasonably sure of certain general conclusions which we have taken the liberty of summarizing as follows: There is a tendency for spontaneous self-fertilization in the genus *Melilotus* to vary with the individual plant. Some species favor autogamy more than others. In general, *M. alba* and *M. coerulea* are more pronounced self-fertilizers than *M. officinalis*. No seed was obtained by selfing *M. dentatus*, *M. suaveolens*, and *M. infestus*. Good seed setting was always obtained by selfing *M. wolgicus*, *M. polonicus* and *M. indicus*. With *M. altissimus*, *M. italicus* and *M. sulcatus* the results varied between plants within each species. Within single plant progenies the results are much more uniform than between unrelated plants.

Self-fertilization in M. alba

Observations over a period of years have given the writers considerable evidence that at least some varieties and strains of *M. alba* consist of a mixture of naturally self-fertilizing and naturally cross-fertilizing plants. These observations may be stated as follows:

(1) When single plant progenies from open-fertilized seed are grown and the plants compared, some of the families show very little variability.

(2) When single plant progenies from first generation self-fertilized seed are grown, a certain percentage of the lines possess a high degree of uniformity in plant týpe, while others are extremely variable.

(3) When single plants are caged to exclude insects, some plants produce much more seed than others. This fact may be seen in the data presented by Kirk (5), Dann (2), and Ufer (8).

(4) Plants grown in a greenhouse which has been thoroughly screened to exclude insects are so variable in seed setting that some of them produce no seed at all, while others produce a pod for practically every flower. We have observed this fact for three seasons.

During the past two years selfed seed has been taken from plants which are able to produce self-fertilized seed spontaneously in different degrees, and progenies from these individuals were grown in order to study the genetics of this character. At this time it is necessary to submit only one piece of evidence to show that clear cut segregation takes place.

In the fall of 1929 sweet clover plants were taken into the greenhouse for various purposes. Among these were two plants from a first generation selfed line. When these blossomed during the winter, one plant produced a large amount of seed by spontaneous self-fertilization, while the other produced no seed except by manipulation of the flowers. Subsequent observation with the other 32 plants of this line showed that these had segregated for this character.

TABLE I

PODS PRODUCED BY HIGH AND LOW SEED SETTING PARENT PLANTS FROM A SINGLE SELFED LINE OF *M. alba*

Plant No.	No. of racemes	Total pods produced	Pods per raceme	Seed setting, %
High seed setting parent plant				
1	18	510	28.34	41
2	17	626	36.82	54
3	15	505	33.67	50
4	16	532	33.25	49
5	21	750	35.71	52
6	15	504	33.60	49
7	13	545	34.23	50
8	21	1395	66.43	98
Low seed setting parent plant				
9	21	3	0.14	0.2
10	21	0	0.00	0.0
11	10	0	0.00	0.0
12	19	6	0.32	0.5
13	19	1	0.05	0.1
14	21	6	0.29	0.4

Selfed seed was secured from the two plants mentioned above, and progenies from each were grown in the season of 1930. Because of limited greenhouse space only 14 plants could be studied in the greenhouse. Six of these came from the non-seeder and eight from the heavy seeder. The amount of seed setting by each plant is given in Table I.

It will be observed from Table I that the low seed setting parent produced only plants which were incapable of natural self-fertilization. On the other hand, the high seed setting parent produced one plant (No. 8) which was completely self-pollinated and naturally self-fertilized. Almost all of the flowers on this plant produced mature pods. The other seven plants of the same family were intermediate with respect to the number of seeds produced, the average being about 49%. From these observations and other data which are still incomplete, we concluded that the character of spontaneous self-fertilization in *M. alba* is inherited and that those plants were heterozygous which were intermediate in seed production.

The data presented in Table I have been selected from inheritance studies (which will be published later), in order to show that the character of natural self-fertilization in white sweet clover exhibits clear-cut segregation, and also because these plants were part of the material which was used in studying the mechanism by which self-fertilization is facilitated or prevented. The flower structure and behavior in relation to seed setting constitutes the chief topic of discussion in this paper, but before proceeding with the subject, it will be necessary to describe the remainder of the material which was studied. This includes several varieties of yellow blossom sweet clover, to which we shall refer briefly in order especially to record a particular case of natural self-fertilization in this species.

Self-fertilization in M. officinalis

While opinions have differed widely as to whether *M. alba* will or will not set seed without artificial treatment or the visitation of insects, all investigators, including the writers, have been agreed that M. *officinalis* is not spontaneously self-fertilized. It is of special interest, therefore, to report an exception to this rule. The variety in question is called "Redfield Yellow", because it was developed at the U.S.D.A. plant breeding station at Redfield, South Dakota. That the plants produce seed by being naturally self-fertilized does not appear to have been reported by those who have given it prominence. This variety is being grown quite extensively at the present time in western South Dakota and adjacent states.

When Redfield Yellow sweet clover was first grown by us about four years ago, we were surprised to see the distinctive appearance and uniformity of plant type. This was observable in the general appearance of stems and foliage, its very late maturity, and characteristic appearance of the inflorescence. The absence of variability in these characters was in sharp contrast to that of other varieties of the yellow flowered species. Not until plants were grown in the greenhouse and brought to maturity in the winter by the aid of electric lights did we observe that approximately 70% of the flowers set seed without artificial manipulation.

The other types of yellow blossom sweet clover which were used in this study included (1) Albotrea, a variety of the common American type, and (2) Zouave, which differs from common yellow sweet clover in producing an upright growth and retaining its foliage late into the fall of the first season. These two types will not produce seed unless the flowers are manipulated artificially.

That the different types of yellow sweet clover mentioned above should be referred to *M. officinalis* is not at all certain, but until more is known with respect to the taxonomy of the genus there seems no good reason for recognizing more than one species of biennial sweet clover with yellow blossoms.

Flower Structure and Pollination in Relation to Seed Setting

Having discovered self-fertilizing white-blossom sweet clover and strains which will not set seed by natural self-fertilization, as well as plants which are intermediate between these extremes, we were much interested in investigating the flower structure and mechanism which might be responsible for the differences in behavior. Having discovered also that Redfield Yellow sweet clover was naturally self-fertilized we were anxious to include in the study several yellow flowered varieties.

In order to provide a ready means of reference to the material which was studied, the different types and varieties have been assigned the following letters:

*A.—*M. alba.* Plants spontaneously self-fertilized, the flowers of which nearly all produce pods (Table I, plant 8).

**B.*—*M. alba.* Plants do not produce seeds normally by self-fertilization (Table I, plants 9 to 14).

**C.*—*M. alba.* About 50% of the flowers produce pods by spontaneous self-fertilization (Intermediate types, Table I, plants 1 to 7).

D.—*M. officinalis* (Variety, "Redfield Yellow"). Plants spontaneously self-fertilized.

E.—*M. officinalis* (Variety, "Albotrea"). Plants do not produce seeds normally by spontaneous self-fertilization.

F.—*M. officinalis* (Variety, "Zouave"). Plants do not produce seeds normally by spontaneous self-fertilization.

In each of the types and varieties (A to F) large numbers were carefully examined in order to determine, if possible, the factor or factors which influence seed setting. We hoped, then, to be able to relate this information to what was known about the actual percentage of seed production under controlled conditions of pollination. Having removed the petals, flowers were observed singly under the microscope.

The results of this study, in which several plants of each type and thousands of flowers were examined, proved very instructive. Several details of flower structure and behavior were noted which appeared to be significant as affecting the tendency of certain types to produce seed by spontaneous self-fertilization

**A, B and C plants belonged to a single first generation selfed line.*

and the actual percentage of pods produced. These observations had to do chiefly with the relative position of anthers and stigmas and the quantity and condition of the pollen. For a long time it seemed impossible to construct any kind of picture which could be related consistently to seed setting percentages of the different types of sweet clover. Besides, information was lacking as to the exact stage of flower development at which the anthers dehisce, because it was not certain whether the anthers had been disturbed when the petals were being removed, resulting in premature liberation of the pollen.

At this stage the idea occurred to us of attempting to render the petals transparent by treating the flowers with some substance that would act as a clearing agent. After many trials with different preparations, a method was devised which was found to be perfectly satisfactory. It was successful not only with white flowers but also with yellow flowers, making the position of stamens, style and pollen grains clearly visible. In the case of yellow petals, most of the coloring disappeared, while the natural color of anthers and pollen was not appreciably affected.

When it is desired to render the flowers transparent in order to observe the normal internal condition at any stage of development, the standard is first removed with a pair of tweezers and the two wing petals clipped off about halfway down. Each flower is then placed on its side on a glass slide. A large number of flowers may be arranged in series and treated at one time. The flowers are then saturated with absolute alcohol for 15 min. by applying one drop at a time to each as often as is necessary. They are then treated for 30 min. with sufficient cedar clearing oil to cover them, and observations are made through the oil. A magnification of 60 was used in these experiments.

With this improvement in technique it was now possible to extend our information to include the exact stage of flower development at which the anthers normally discharge their pollen. The natural distribution of pollen also, and its position in relation to the stigma, was clearly evident. These facts proved to be of first importance. So successful indeed was this method of examination that we decided to duplicate all previous observations and measurements.

Several factors have been referred to already which may conceivably affect seed setting in sweet clover, and there are still others, not yet described, which may be added to the list. These will now be considered separately, and as far as possible an attempt will be made to interpret the behavior of each variety with respect to seed production in terms of the special combination of factors which it exhibited.

Position of stigma in relation to the anthers. The stamens in different plants of *M. alba* and in plants representing different varieties of *M. officinalis* were found to vary greatly in length. In flowers with short stamens the style protruded a considerable distance beyond the highest anther: This condition was typical in the case of all plants designated B. These produced practically no pods unless the flowers were manipulated artificially. "A" plants of the same selfed line had long stamens, the anthers were as high as the stigma,

and practically all flowers produced pods. "C" plants of the same selfed line had stamens of intermediate length, and all of these plants produced about 50% of the possible number of pods (see Fig. 2). So far as *M. alba* is concerned, therefore, this character appeared to have some significance in the material which was studied.

In order to show the degree of variability within each of the types A, B and C in *M. alba*, measurements were made on 100 flowers of each plant. Instead of measuring the length of the filaments, which presented certain difficulties, the more significant determination was made of the distance from tip of style to highest anther. This information is presented graphically in Fig. 1. The curve (A) for flowers with long stamens would have been as symmetrical as the others had not the anthers in many of the flowers protruded beyond the style.

FIG. 1. *Class frequencies (based upon length of styles projecting beyond anthers) in flowers of plant segregates in a selfed line of white sweet clover (Melilotus alba). (A) Naturally self-fertilized flowers with long stamens. (B) Flowers with short stamens and not naturally self-fertilized. (C) Flowers with stamens of intermediate length. About 50% were naturally self-fertilized.*

With respect to varieties of *M. officinalis* which were studied for length of stamens, the condition was very interesting, since there was no such consistent relation to seed production as was found in *M. alba*. Redfield Yellow, which produces a high percentage of pods by natural self-fertilization, had very short filaments similar to the "B" type plants of *M. alba*. On the other hand, plants of the variety Albotrea had very long filaments so that the anthers were adjacent to the stigma and frequently they were above it. In this respect these corresponded to the "A" type plants of *M. alba*, but unlike the latter they failed to produce pods when the flowers were undisturbed. The third variety, Zouave, had very short stamens and did not produce pods by natural self-fertilization. This variety, therefore, did not violate the rule which seemed to apply for the plants of *M. alba* which were studied.

With respect to the relative position of anthers and stigma, we can say only that in the plants of *M. alba* which were studied the close proximity of anthers and stigma seemed to facilitate self-fertilization, but that this assumption did not hold as far as *M. officinalis* was concerned. Obviously, there must be other factors which play a part.

Stage of flower development when the pollen is liberated from the pollen sacs. In all of the material which was studied it was found that at least some of the pollen sacs had discharged their pollen within the flower just preceding the

unfolding of the bud. In other words, one or two and sometimes three or four unopened flowers, immediately above the last blossom to expand, were found almost invariably to contain some free pollen. Obviously this fact only served to complicate the problem. It is interesting to note that its discovery could not have been arrived at except by the method of making the petals transparent. For reasons which need not be stated it is certain that the treatment did not contribute to the observed result.

Distribution of pollen at the time when the flowers expand. Careful observations were made on all of the material in order to determine the exact distribution of pollen within the flower. For this purpose the last blossom to open on the raceme was chosen. In other words, the flowers selected were those only which are the last to open and immediately adjacent to the unexpanded buds.

The observed distribution of pollen within the flowers, taken in conjunction with determinations on the relative position of anthers and stigmas, appears to be very significant, and throws a good deal of light on the problem of spontaneous self-fertilization in sweet clover. Considering first the A, B and C type plants of *M. alba*, it was found that the percentage of flowers having pollen on the stigma was in exact agreement with the percentage of pods formed, the latter being based on total number of flowers. Observations were made on 100 flowers per plant. The stigmas of "A" type plants invariably were covered with pollen. In about 50% of the cases, flowers of "C" type plants had pollen on the stigmas. Very rarely, however, was it possible to find flowers on "B" type plants in which a single pollen grain was found on the stigma. In this connection it should be stated that all three types would set seed freely when the flowers were artificially manipulated.

In the yellow flowered species only two of the three varieties, namely Redfield Yellow and Zouave, showed the same exact agreement between seed setting and distribution of pollen which was observed in *M. alba*. In Redfield Yellow, approximately 70% of the flowers had pollen grains on the stigma and the percentage of pods was about the same. In no case was pollen found on the stigmas in flowers of the variety Zouave, and no seed was produced by natural self-fertilization. But Albotrea, in this case, proved to be the exception to the rule, since this variety produced no pods, although pollen had been deposited on the stigma in 95% of the cases. With respect to distribution of pollen, therefore, it may be stated that a perfect correlation exists in five of the types and varieties which were studied between percentage of seed setting and percentage of flowers which had pollen grains in contact with the stigmas. The Albotrea variety proved to be a notable exception in this respect.

Fig. 2 shows the average condition which was found in flowers of the different plant types which were studied with respect to distribution of pollen as well as the relative position of stamens and styles. The drawings were made with a camera lucida attachment and with a magnification of about 120 diameters.

Size of keel. Differences were observed with respect to flower size in different varieties. Size of flower determines the amount of space within the upper portion of the keel above the anthers. The relative sizes are indicated in Table III. This factor appeared to have significance in the case of Redfield

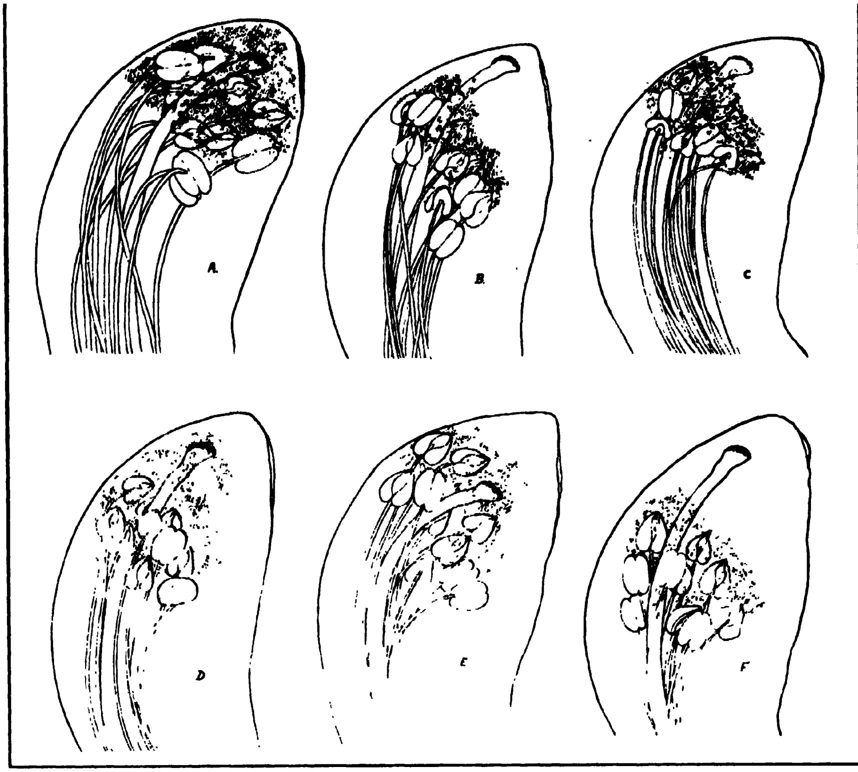


FIG. 2. *Camera lucida drawings showing the average condition within the keel when sweet clover flowers first open with respect to length of stamens and distribution of pollen. A, B, C Plant segregates in a selfed line of M. alba. D, E, F—Varieties of M. officinalis; Redfield Yellow, Albotrea, and Zouave, respectively. A and D are naturally self-fertilized. B, E and F will not set seed unless the flowers are manipulated. C is naturally self-fertilized in about 50% of the cases.*

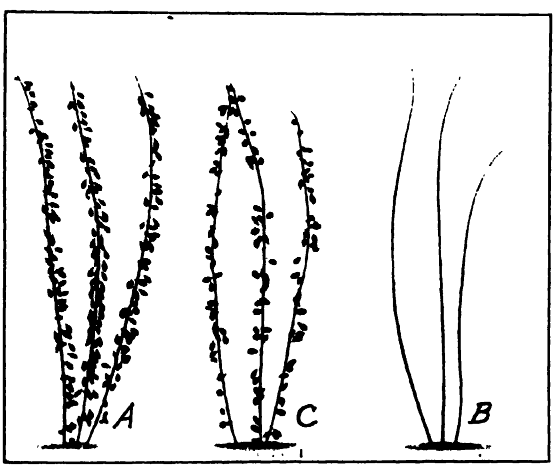


FIG. 3. *Racemes from plant segregates in a selfed line of white sweet clover, M. alba, showing the typical numbers and distribution of pods at maturity. (A) Naturally self-fertilized. (B) Not naturally self-fertilized. (C) Fifty per cent of the flowers naturally self-fertilized.*

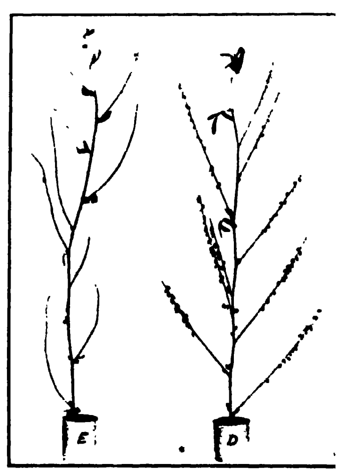


FIG. 4. *Yellow blossom sweet clover, M. officinalis, showing seed setting in two varieties. (D) Redfield Yellow; (E) Albotrea.*

Yellow sweet clover, since it appeared to be the only explanation why the pollen mass, in flowers of this variety, was nearly always heaped up beyond the anthers until the highest pollen grains came in contact with the stigmas. The mass of pollen grains always gave the appearance of having been pushed upwards in close quarters. Whether this is the explanation of what actually takes place, it is impossible to say, but the peculiar distribution of pollen in this variety was very characteristic (see Fig. 2).

Amount of pollen. This is a factor which appears to be of considerable importance if we may judge by the striking differences which were found between the different types and varieties which were studied. As between flowers and plants within each type, there was very little variation.

Plants of *M. alba* which produced seed by natural self-fertilization ("A" type, with long stamens) invariably had flowers with large amounts of pollen. "C" type plants of *M. alba*, which were intermediate with respect to length of stamens and seed setting, in general produced an intermediate quantity of pollen. "B" type plants of *M. alba*, which had short stamens and did not produce seed by natural self-fertilization, invariably produced a very limited supply of pollen.

In the yellow flowered species, Redfield Yellow, with short stamens and high seed setting, had a large quantity of pollen. Zouave and Albotrea varieties, on the other hand, with very short and very long stamens respectively and neither of which produced seed by self-fertilization, were intermediate with respect to quantity of pollen.

With reference to quantity of pollen, therefore, it may be said that large quantities were found to be closely associated with high seed setting, and small amounts with low seed setting, under the conditions of the experiment. Quantity of pollen was not associated with length of stamens. It was not possible in these studies to attach any special significance to this factor, but the facts were so striking as to be of special interest, and call for further study.

Condition of pollen. Perhaps the most interesting observation which was made in connection with spontaneous self-fertilization in sweet clover had to do with germination of the pollen grains in the flower. During the investigation it was early observed that when flowers were tripped with the flat end of a wooden toothpick certain plants discharged a large quantity of pollen, the grains of which tended to cling together in a mass. This was characteristic of "A" type plants of *M. alba* and of the variety Redfield Yellow, both of which produced an exceptionally high percentage of pods. The other four types studied produced either a medium or very small amount of pollen, and this was always dry and powdery. These two conditions of pollen gave the impression that some plants produced sticky pollen and others, pollen which was just the reverse. Examination under the microscope, however, disclosed the fact that this tendency for the pollen grains to cling together in a mass was due to their having germinated. The mass consisted not only of pollen grains but of pollen tubes which served to hold them together.

It was discovered further (by observing the flowers after they had been rendered transparent) that in the two cases referred to above, the pollen grains

invariably had germinated *en masse* before the flowers had opened. This would seem to be a very remarkable adaptation to insure self-fertilization. We arrive at the conclusion, therefore, that plants of the "A" type in *M. alba* and plants of the Redfield Yellow variety in *M. officinalis* are completely and spontaneously self-fertilized. Cross pollination by means of insects would seem to be a practical impossibility.

In the flowers of the other types of sweet clover which were studied, those having so-called "dusty" pollen, germinated pollen grains were never found. It should be stated that attempts to germinate such pollen grains in water were always successful, but only with a small proportion of them. This proved that they did not altogether lack viability. Presumably the percentage germination would have been quite satisfactory in a more favorable medium. This is being investigated.

Another point in this connection should be recorded because it strengthens the assumption that certain types of plants are normally self-fertilized, and because it is useful information in connection with artificial crossing in sweet clover. In the case of "A" type plants in *M. alba* and plants of the Redfield Yellow variety, both of which produced germinating pollen in the unopened flowers, it was practically impossible except by drastic methods to remove all of the pollen from the surface of the stigmas. On the other hand, flowers of plants which had "dusty" pollen could be taken after they had opened, the petals removed, and the stigmas completely freed from pollen by simply blowing on them. This may be done with the breath or by means of a dental syringe. What has been stated is especially true of the "B" type plants in *M. alba* which did not produce pods by natural self-fertilization. Pollen was rarely found in contact with the stigmas, even after the petals were removed from fully opened flowers. With these plants emasculation is a simple procedure which is likely to be highly efficient by any method. On the other hand, emasculation is very difficult and highly unreliable with any method in the case of those types where the pollen is germinated before the flowers open, unless the latter are taken at a very early stage of development.

Receptivity of the stigma. The problem still remained to be explained why the variety Albotrea of the yellow flowered species would produce practically no seed, although pollen grains were found in contact with the stigmas in 95% of the flowers, and this at an early stage of development before the petals had expanded. The same problem existed to a lesser degree with respect to "B" type plants of *M. alba* and plants of the variety Zouave, either of which would produce only a comparatively small amount of seed when the petals were removed and the stigmas dusted with pollen from the same plant. Since some of the pollen at least was known to be viable, it was natural to suspect that the stigmas in some manner were not receptive.

Plants which are not capable of spontaneous self-fertilization must depend for their fruitfulness on the visitation of insects. It is possible that when honey bees, which are the most important pollinators of sweet clover, visit the flowers, the stigmas are more or less scarified by coming in contact with the body of these insects. Since no other circumstance suggested itself which was

likely to affect the stigmas of sweet clover flowers, an experiment was planned to test the validitiy of this hypothesis.

Several racemes were selected on each of two plants of the three varieties in question, and one other plant of *M. officinalis* designated "X". The petals were carefully removed from a number of freshly opened flowers. On ten racemes of each type the flowers were dusted with pollen from the same plants, care being taken to make sure by examination with a lens that an abundant supply of pollen grains were adhering to the stigmas. Another group of ten racemes had the stigmas of each flower crossed back and forth with a small camel's-hair brush. The third group of ten racemes was treated by rubbing the stigmas with the flat end of a toothpick. Pollen was applied after each operation. The result of this experiment is presented in Table II, which gives the number of flowers treated and the number of pods produced in per cent for each treatment and each variety.

TABLE II

EFFECT OF SCARIFICATION ON RECEPTIVITY OF THE STIGMAS IN TERMS OF SEED SETTING IN VARIETIES OF SWEET CLOVER WHICH DO NOT PRODUCE SEED NORMALLY BY SPONTANEOUS SELF-FERTILIZATION

Variety	No. of racemes	No. of flowers	Method of scarifying the stigma	No. of pods produced	Per cent of flowers which produced pods
B type plants					
M. alba	10	125	None	19	15.2
M. alba	10	110	Soft brush	20	18.2
M. alba	10	90	Toothpick	64	71.1
Zouave					
M. officinalis	10	142	None	9	6.3
M. officinalis	10	141	Soft brush	15	10.6
M. officinalis	10	152	Toothpick	100	65.8
Albotrea					
M. officinalis	10	162	None	6	3.7
M. officinalis	10	158	Soft brush	7	4.4
M. officinalis	5	74	Toothpick	32	43.2
"X"					
M. officinalis	10	125	None	22	17.6
M. officinalis	10	115	Soft brush	37	32.2
M. officinalis	10	110	Toothpick	97	88.2

It is evident from the data presented in Table II that scarification of the stigmas with a toothpick was effective in making the stigmas receptive. The results were not very different for each of the three varieties. Apparently the treatment with a camel's-hair brush was not sufficiently severe to make a significant difference in the number of pods produced.

Seven factors have been described which appear to be more or less associated with the degree of spontaneous self-fertilization in sweet clover. These are tabulated in Table III in order to show the precise combination of characters which were characteristic of the plants in each of the types and varieties which

were studied. A large number of flowers on each plant were examined, but the number of plants in each variety was necessarily very limited. Those which were studied happened to possess the same characteristics, but it is very improbable that these are the only combinations of factors which would have been found if a larger number of individual plants had been examined.

TABLE III

FACTORS WHICH APPEAR TO INFLUENCE THE DEGREE OF SPONTANEOUS SELF-FERTILIZATION IN TYPES AND VARIETIES OF SWEET CLOVER

Character	Types of *M. alba*			Varieties of *M. officinalis*		
	A Self-fertilized	B Not self-fertilized	C Inter-mediate	D Redfield Yellow. Self-fertilized	E Albotrea. Not self-fertilized	F Zouave. Not self-fertilized
1. Percent seed setting	98	0.05	49	70	None	None
2. Length of stamens	Long	Short	Inter-mediate	Short	Long	Short
3. Liberation of pollen	Unopened flower	Unopened flower	Unopened flower	Unopened flower	Unopened flower	Unopened flower
4. Distribution of pollen	On stigma 100%	On stigma 0.25%	On stigma 50%	On stigma 70%	On stigma 95%	On stigma 0.0%
5. Size of keel cavity	Small	Small	Small	Very small	Large	Very large
6. Amount of pollen	Abundant	Scarce	Medium	Abundant	Medium	Medium
7. Condition of pollen*	Ger-minated	Not ger-minated	Not ger-minated	Ger-minated	Not ger-minated	Not ger-minated
8. Receptivity of stigma*	Receptive	Not receptive	Receptive	Receptive	Not receptive	Not receptive

***Data in these cases were obtained at an early stage of development just at the time when the flowers first open. In types A and D the pollen grains were germinated before the flowers opened.**

Discussion

The most significant fact brought out by this investigation is that certain plants of sweet clover are normally self-pollinated and spontaneously self-fertilized. In some cases cross-pollination is impossible, or at least negligible. It is fairly clear also that those peculiarities which characterize a plant of this type are inherited, and may therefore be utilized in breeding. The information which we have at the present time seems to indicate that the essential factors may be transmitted in a comparatively simple manner.

The significance of complete spontaneous self-fertilization as an inherited character in sweet clover is very important and quite obvious. Improved new varieties which are homozygous for this character may be grown without

danger of contamination with foreign pollen, and the superior qualities which they possess can be reproduced indefinitely, as is the case with most cereals. Indeed, the percentage of natural crossing may be less than in some cereals.

Natural self-fertilization in sweet clover will be a great asset in breeding for disease resistance. This crop is vulnerable to a fairly large number of destructive pathogenes, and it is becoming more and more evident that breeding for disease resistance will be one of the major problems with this crop. If selfed lines which are completely self-fertilized can be secured without too much effort, the task becomes very much less difficult. It is one thing to produce disease resistance, but quite another to retain the advantage of such a quality after it has been secured.

We have believed for a long time that plants which are naturally self-fertilized are of fairly frequent occurrence in *M. alba*, for the reasons stated earlier in this paper. Definite information has now been submitted to show that such plants do occur. The proportion of such plants will doubtless vary with different varieties of the white flowered species, but our observations suggest that spontaneous self-fertilization is associated more especially with the mid-tall, early maturing sorts such as are represented by the varieties "Arctic" and "Grundy County". There is reason to believe that the "Arctic" variety contains a considerable proportion of naturally self-fertilizing plants. Several of these have been isolated.

In view of the enormous advantage which a self-fertilizing variety would have from the standpoint of pure seed production, it would seem that plant breeders who are working with this crop should fully canvass the possibilities of spontaneous self-fertilization with the ultimate ambition to make all new productions homozygous for this character.

So far as *M. officinalis* is concerned, spontaneous self-fertilization seems to be of rare occurrence. During many years' work with several distinctive sorts of yellow flowered sweet clover, we found no indication that plants with this character exist until it was observed in the variety "Redfield Yellow".

Preliminary observations on the factors which appear to influence self-fertilization in sweet clover were made in connection with crossing work when the flowers were being emasculated by the "suction" method. This method was described by Kirk (6) and consists of removing the petals of fully opened flowers, after which the pollen is removed by suction through a small glass nozzle, the latter being attached by a rubber hose to an aspirator pump. The "suction" method has been criticized recently by Hackbarth (3) as being less effective than the water-jet method for emasculating alfalfa described by Oliver (7). It would appear that the facts reported herein offer a satisfactory explanation for the divergent results obtained by different investigators and show why the suction method can be very successful with certain types of plants but quite unsuccessful with others.

With respect to ease of emasculation, at least several different plant types may be distinguished as follows: (1) Plants with short, medium or long stamens, "dusty" pollen and unreceptive stigmas. These can be emasculated with ease and a high degree of certainty by almost any method. Very satisfactory

results were obtained by simply removing the petals and blowing off the pollen with a forced breath. (2) Plants with short stamens, "dusty" pollen, and receptive stigmas. With these the "suction" method is quite successful because the pollen grains do not come in contact with the stigmas unless the flowers are manipulated. In these cases also, a forced breath will remove the pollen grains quite effectively, and the "selfs" will be few in number. (3) Plants with long stamens, "dusty" pollen and unreceptive stigmas. These can be emasculated successfully with air or water pressure. If the "suction" method is used great care must be taken not to injure the stigmas, because if this takes place pollen grains may become strongly attached and self-fertilization is likely to occur. (4) Plants with long stamens and receptive stigmas. Whatever method is used, plants of this type must be emasculated at a very early stage of development before the flowers open. In these cases the "suction" method is of little value.

The difficulty of successful emasculation in the fourth type listed above is still greater if the pollen grains normally have germinated before the flowers open. Not only the "suction" method, but also the water-jet method and emasculation with a needle have proven very inefficient in our experiments with plants that possess these peculiarities. Germinated pollen adheres to the stigma much more tenaciously than "dusty" pollen, presumably because they are held fast by germ tubes which have penetrated the tissues.

In our original experiments with the suction method we were unaware of the differences in flower structure and behavior which exist between different plants of sweet clover. The data which were presented (6) seemed to be sufficiently conclusive to justify a favorable report. On the other hand, all of the experiments were made with a comparatively small number of plants and these may have included only types (1) and (2), and not types (3) and (4).

Acknowledgment

The authors which to express their thanks to Mr. Frank Rose, Field Assistant, who has contributed much to the success of this investigation both in the field and in the laboratory.

References

1. Coe, H. S. and Martin, J. N. Sweet clover seed. U.S. Dept. Agr. Bull. 844. 1920.
2. Dann, B. Uber die Befruchtungsverhaltnisse der Bastardluzerne (*Medicago media*), anderer *Medicago*-Arten und Steinklee (*Melilotus*). Z. Pflanzenzuchtg. 15: 366-418. 1930.
3. Hackbarth, J. Künstliche Kreuzungsmethoden bei Steinklee und Luzerne. Der Zuchter, 2: 354-358. 1930.
4. Kirchner, O. Uber die Wirkung der Selbstbestanbung bei den Papilonaceen. Naturwissenschaften, 3: 1-16. 49-64, u. 97 bis III. 1905.
5. Kirk, L. E. Self-pollination of sweet clover. Sci. Agr. 6: 109-112. 1925.
6. Kirk, L. E. Abnormal seed development in sweet clover species crosses. A new technique for emasculating sweet clover flowers. Sci. Agr. 10: 321-327. 1930.
7. Oliver, C. V. Alfalfa seed production: Pollination studies, U.S. Dept. Agr. B.P.I. Bull. 75. 1914.
8. Ufer, M. Untersuchungen über die Befruchtungsverhältnisse einiger Melilotusarten (Steinklee). Der Zuchter, 2: 341-354. 1930.

CHEMICAL CHANGES IN NITROGEN FRACTIONS OF PLANT JUICE ON EXPOSURE TO FROST[1]

By R. Newton[2], W. R. Brown[3] and J. A. Anderson[4]

Abstract

Exposure of the press-juice of unhardened winter wheat plants to frost caused a decrease in coagulable protein content, an increase in amino nitrogen content and an increased sensitivity to hydrolysis by dilute sodium hydroxide. Added sugar reduced the amount of change. No change resulting from freezing could be demonstrated in the inorganic nitrogen fractions. It is suggested that protein splitting as found in hardened plants may be the result of frost rather than an adaptation against it, and that the value of sugar as a protection to winter plants may lie partly in its ability to delay this action.

Introduction

The splitting of proteins during the hardening process in the autumn to simpler, less easily precipitated forms has been held by Schaffnit (4) and Harvey (1) to be an adaptation of winter plants against frost injury. Harvey found that the amino-acid content of cabbage leaves increased on hardening, and concluded that the protein cleavage thus indicated was the principal effect of the hardening process. Though not expressly stated, the inference from his paper is that the plants analyzed were never exposed to frost during hardening in a cold-frame. The increase of amino nitrogen we have fully verified in work with winter wheat plants (2), but have observed that the most pronounced increase occurs after the advent of sharp night frosts.

In the course of work on the frost precipitation of the proteins of plant juice (3), it was noticed that the nitrogen fractions seemed to be slightly altered during exposure to frost. The questions arose as to whether the frost had caused some chemical change in the proteins, and whether protein splitting as observed in winter plants was an effect of frost rather than an adaptation against it. A closely related question concerned the possibility that the protective value of sugar might lie in its power to delay frost cleavage.

Experiments looking to an answer to these questions were carried out in 1925, and repeated in 1927 for the purpose of checking and extending the results.

Materials and Methods

The winter wheat plants used were grown in the greenhouse, to obtain tissue in an unhardened condition in which the proteins would be sensitive to frost. The press-juice was extracted and handled as previously described (3).

[1] *Manuscript received June 10, 1931.*

Contribution from the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada.

[2] *Professor of Field Crops and Plant Biochemistry, University of Alberta.*

[3] *Formerly postgraduate student, University of Alberta.*

[4] *Junior Research Biologist, National Research Laboratories of Canada.*

In 1925, the nitrogen of the fresh juice and of the juice, with and without added sugar, which had been stored in an ice-salt bath at $-7°$ C., was classified into four fractions:

(1) heat-coagulable protein nitrogen,
(2) non-coagulable organic nitrogen,
(3) nitrate-nitrite nitrogen,
(4) ammonia nitrogen.

Coagulable nitrogen was determined by pipetting triplicate 10-cc. samples of press-juice into test tubes, adding to each 1 cc. $N/1$ sodium acetate and $N/1$ acetic acid, and immersing in a boiling water bath for 30 min., with frequent stirring. The juice was then filtered and the coagulum washed with 150 cc. of hot, distilled water. The filter paper and contents were then transferred to a Kjeldahl flask and the nitrogen determined by the Kjeldahl-Gunning-Arnold method.

The combined nitrate-nitrite and ammonia nitrogen was determined on the filtrate, made up to 250 cc. in a Kjeldahl flask, by Strowd's modification of the Devarda method (5), but substituting caprylic alcohol for paraffin as an anti-foam agent. Paraffin occluded carbon, and made complete digestion of the residue impracticable.

Non-coagulable organic nitrogen was determined by the Kjeldahl-Gunning-Arnold method, on the residue from the Devarda distillation, in the same flask.

Ammonia was determined on a fresh sample of juice, by distilling after diluting with water to 250 cc. and adding 2.5 gm. of sodium hydroxide, as in Strowd's modification of the Devarda distillation. The nitrate-nitrite fraction was obtained by subtracting the ammonia value from the combined value noted above.

In 1927, the following modifications of these methods were made. Determinations of coagulable nitrogen were carried out in quadruplicate (instead of triplicate). The determination of non-coagulable organic nitrogen was discarded, and a determination of amino-acid nitrogen by the Van Slyke method, on an aliquot of the undiluted filtrate from the coagulable nitrogen determination, substituted as a better index of changes in organic nitrogen fractions. The combined nitrate-nitrite and ammonia fractions were determined as before by the modified Devarda distillation of the filtrate from the coagulable nitrogen determination, but the ammonia was determined on aliquots of the same filtrate instead of on samples of fresh juice. Also, the ammonia distillation was carried out by the Van Slyke method, at 20 mm. pressure and 45° to 50° C. for 30 min., the filtrate being first saturated with calcium hydroxide. The amounts of ammonia found by this method were so small that to measure them satisfactorily it was necessary to use 100 cc. aliquots of undiluted filtrate. These were obtained by refluxing 250 cc. of juice in a boiling water bath for 30 min. and filtering.

Results of 1925 Experiments

The two series of results obtained in 1925 are included in the first half of Table I. They show that exposure to frost caused a decrease in the coagulable

nitrogen and apparently also in the nitrate-nitrite nitrogen, with a corresponding increase in non-coagulable and ammonia nitrogen. The changes were less in the sugar-protected juice.

TABLE I

CHANGES IN NITROGEN DISTRIBUTION OF PRESS-JUICE ON FREEZING

Wheat variety and nitrogen fractions	Mg. nitrogen in 10 cc. juice		
	Fresh juice	Frozen 20 hr. at $-7°$ C.	
		Juice+8% sucrose	Juice only
Minhardi, 1925			
Coag. protein nitrogen	44.5	43.3	42.3
Non-coag. organic nitrogen	11.5	13.3	13.5
Nitrate-nitrite nitrogen	11.5	12.0	10.7
Ammonia by distn. with NaOH	3.7	3.5	4.6
Fulcaster, 1925			
Coag. protein nitrogen	32.5	30.8	30.7
Non-coag. organic nitrogen	9.8	11.0	11 1
Nitrate-nitrite nitrogen	13.3	12.3	11.7
Ammonia by distn. with NaOH	2.5	3.5	3.6
Minhardi, 1927			
Coag. protein nitrogen	22.9	22.6	22.3
Amino acid nitrogen	2.44	2.61	2.75
Nitrate-nitrite nitrogen	14.71	14.27	15.31
Ammonia by distn. with NaOH	0.90	1.21	1.30
Ammonia by Van Slyke distn.	0.15	0.14	0.15
Turkey, 1927			
Coag. protein nitrogen	34.2	33.3	33.2
Amino acid nitrogen	3.05	3.30	3.63
Nitrate-nitrite nitrogen	14.15	14.50	13.44
Ammonia by distn. with NaOH	1 17	1.10	1.17
Ammonia by Van Slyke distn.	0 32	0.24	0.21
Fulcaster, 1927		Frozen 6 hr. at $-7°$ C.	
Coag. protein nitrogen	20.4	20.8	20.4
Amino acid nitrogen	3 30	3.04	3.32
Nitrate-nitrite nitrogen	14.43	14.64	14.71
Ammonia by distn. with NaOH	1.03	0.82	0.81
Ammonia by Van Slyke distn.	0.11	0.09	0.10

The changes in the organic fractions were interpreted as evidence that protein splitting is at least partly the result of exposure to frost, rather than an adaptation against frost injury. The smaller changes in the presence of sugar suggested that the protective activity of the latter may be in part chemical. The apparent changes in the inorganic fractions were not looked for, and the experiments of 1927 included a critical examination of the methods which led to the earlier result.

It will be seen in the first half of Table I that if the organic fractions are bulked together and the inorganic fractions treated likewise, that no appreciable

change has taken place in the totals. What is required therefore is a verification of the protein splitting which would account for the change of a small part of the coagulable to the non-coagulable form, and an explanation of the apparent reduction of nitrate-nitrite to ammonia.

Results of 1927 Experiments

The results of the 1927 experiments substantiated the change in the organic fractions, in the two cases where the juice was frozen 20 hr. at −7° C. The coagulable protein fraction was slightly decreased by the freezing, and an increase in amino-acid nitrogen afforded direct proof of protein splitting. As before, the changes were less in the portions of juice to which sugar had been added.

In the third case, in which the juice was exposed to frost only 6 hr., the changes if any were not measurable by the methods used.

Before discussing the inorganic fractions, the methods of determining them will be examined. In Table II are given the data for determinations of the combined nitrate-nitrite-ammonia nitrogen, the ammonia nitrogen, and the nitrate-nitrite nitrogen by difference. The methods which yielded the results shown in the first two columns of figures, differed only that in the case of the first column the solution of 250 cc. was heated with a 1 gm. of Devarda alloy for 1 hr. before distillation. The concentration of sodium hydroxide was the same in both cases. These were the methods of 1925, but they were now applied to the coagulable nitrogen precipitate as well as to the filtrate and the fresh juice.

TABLE II

EFFECT OF HYDROLYSIS IN DETERMINATIONS OF AMMONIA AND OF NITRATE-NITRITE BY THE DEVARDA METHOD

Juice from leaves of Khapli wheat	Mg. nitrogen in 5 cc. juice		
	Nitrate-nitrite-ammonia	Ammonia	Nitrate-nitrite by difference
Coag. nitrogen precipitate	1.55	1.75	—
Coag. nitrogen filtrate	3.92	0.80	3.12
Total	5.47	2.55	2.92
Fresh juice	4.72	2.32	2.40

If the method actually determined only nitrate-nitrite nitrogen and inorganic ammonia, it would not be expected to find any in the precipitate, since all the salts should be washed into the filtrate. The data show that in fact nearly 70% of the ammonia found in the fresh juice was due to the hydrolysis of amide and amine groups in the coagulable protein. The discrepancy between the sum of the amounts found in the precipitate and filtrate, and that found directly in the fresh juice, may be due to changes in the composition of

the proteins during the boiling necessary to coagulate them or to unequal hydrolysis during heating with the alloy. In either case it emphasized the empirical nature of the method.

Returning now to the lower half of Table I, it will be seen that in 1927 the ammonia distillations were carried out by two methods: with 1% sodium hydroxide as in 1925, and by the Van Slyke method. The latter method is commonly supposed to determine all free ammonium salts but no organic groups. It showed no increase in ammonia as a result of freezing. Indeed the amounts found are so small they may well be due to slight hydrolysis during heat coagulation. Attempts to determine ammonia on the fresh juice by this method failed owing to the large amount of juice required and the consequent impossibility of preventing frothing by any ordinary antifoam methods.

The sodium hydroxide distillation, like the foregoing, was carried out on the filtrate from the heat coagulable protein (rather than on the fresh juice as in 1925), and in the Minhardi juice showed an increase in the ammonia fraction on freezing. It was not expected to find much if any increase in this fraction determined on the filtrate, since the data in Table II indicate most of the ammonia to have come from hydrolysis of the coagulated protein. As a measure of true ammonia the figures must of course be rejected, but together with the similar results in 1925 they may be accepted as further proof of the breaking down of proteins during freezing. This process sets free amide and amine groups which are hydrolyzed by the sodium hydroxide and determined as ammonia.

Since the concentration of the inorganic fractions was very small and the methods available far from perfect, it cannot be said with certainty that freezing caused no changes in them. It is clear, however, that the apparent changes found in 1925 were mainly due to the error involved in determining ammonia by distilling fresh juice with 1% sodium hydroxide. There was, as already pointed out, no change in the sum of the nitrate-nitrite and ammonia fractions, and since the nitrate-nitrite was found by difference, the establishment of relative constancy in the ammonia necessarily eliminates the apparent variability in both. But the fact that the apparent changes in the inorganic fractions were due in reality to frost-induced changes in the organic fractions, whereby amide and amine groups were exposed to hydrolysis in the subsequent determination of ammonia, is of significance from the point of view of frost effects.

Conclusion

The results on the whole seem to justify the conclusion that frost causes some cleavage of the proteins of wheat leaf juice, as reflected in a decreased content of coagulable protein, an increased sensitivity to hydrolysis by dilute sodium hydroxide, and an increased content of amino nitrogen. The experiments do not afford proof that amino nitrogen may not increase at hardening temperatures without frost, but indicate that the protein splitting held by some investigators to be an adaptation against frost may be partly at least merely the result of frost. It is to be noted also that in a recent paper (3) it

has been shown that precipitation of the proteins of press-juice, comparable to frost precipitation, may occur at 0° C. without freezing. The precipitating action of cold, with or without ice formation, may be connected with protein splitting under the same conditions.

The action of sugar added to the juice, in reducing the amount of change by frost, seems highly significant in view of the widely recognized role of sugars in plants as a factor in frost resistance.

In this laboratory we have found that protein splitting, as indicated by amino nitrogen content, occurs in the late autumn in equal degree in all varieties, without regard to their respective winter hardiness. This lends color to the view that it is the result of frost, since all varieties were exposed to the same weather conditions. Sugar accumulation is also the result of low temperatures, but in this case varieties exhibit different levels corresponding in a general way with their relative winter hardiness, thus suggesting that it is a true adaptation against frost.

References

1. HARVEY, R. B. Hardening process in plants and developments from frost injury. J. Agr. Research, 15: 83-112. 1918.
2. NEWTON, R. and BROWN, W. R. Seasonal changes in the composition of winter wheat plants, in relation to frost resistance. J. Agr. Sci. 16: 522-538. 1926.
3. NEWTON, R. and BROWN, W. R. Frost precipitation of proteins of plant juice. Can. J. Research, 5: 87-110. 1931.
4. SCHAFFNIT, E. Studien über den Einfluss niederer Temperaturen auf die pflanzliche Zelle. Mitt. Kaiser-Wilhelm Inst. Landw. Bromberg, 3: 93-144. 1910.
5. STROWD, W. H. The determination of nitrites and nitrates in plant-tissue. Soil Science, 10: 333-342. 1920.

CATALASE ACTIVITY OF WHEAT LEAF JUICE IN RELATION TO FROST RESISTANCE[1]

By R. Newton[2] and W. R. Brown[3]

Abstract

The catalase activity of press-juice of winter wheat leaves collected from the field at any time during the late summer and fall was directly related to the winter hardiness of varieties.

Introduction

Catalase activity has been quite widely used as a measure of the activity or vitality of tissues. Heinicke (4) states with regard to apple leaf tissue, "it is very probable that the ability to decompose hydrogen peroxide is a more sensitive measure of the metabolic status of the tissue than the usual chemical analysis." Appleman (1) reported catalase activity to be closely correlated with respiratory activity in potato tubers, while Burge (2) concluded that it was associated with oxidative processes in the animal body. Leggatt (5) has recently investigated its relation to seed viability, and has contributed a good review of the literature.

Investigations on winter-hardiness (6, 7) have shown that sugar disappears gradually from the leaves of winter wheat plants during the winter, the rate of loss being somewhat greater in non-hardy varieties. Consequently, the protective resource represented by the sugar is diminished at a corresponding rate. The presumption is that the rate of loss is connected with the rate of respiration. At the time the experiments reported in this paper were carried out, suitable facilities for low-temperature respiration measurements were not available. However, on the evidence of other workers such as Appleman and Burge, cited above, there seemed reason to expect that the respiratory activity of the tissue might be reflected to some degree in the catalase activity of the press-juice. Accordingly a series of determinations was made on the press-juice of plants collected from the field at progressive dates and handled as already described (7). Of the three wheat varieties used, Minhardi is classified as winter-hardy, Turkey as semi-hardi, and Fulcaster as non-hardy.

Method

The determination of catalase activity was carried out quite simply, using a Van Slyke amino nitrogen apparatus, as suggested by Harvey (3). The leaves were dusted with precipitated calcium carbonate before grinding, so that acids destructive to the catalase might be neutralized as soon as liberated. The press-juice was diluted at once with 10 parts of distilled water, as in this dilution its activity will remain unchanged for many hours, whereas the activity of

[1] *Manuscript received June 13, 1931.*
Contribution from the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada.

[2] *Professor of Field Crops and Plant Biochemistry, University of Alberta.*

[3] *Formerly postgraduate student, University of Alberta.*

undiluted juice gradually diminishes. A convenient quantity of the diluted juice, with sufficient water to make to 20 cc., was introduced into the deaminizing bulb through the top funnel, and 10 cc. of Oakland Dioxygen, neutralized with calcium carbonate, added through the side burette at the moment of beginning the determination. A constant rate of shaking was maintained by the use of an electric motor. The time required to liberate constant increments of oxygen was measured with a stop watch. All solutions employed were kept in a water-bath at 20° C., and the laboratory was maintained approximately at this temperature. Without special appliances it was found possible to restrict fluctuations in room temperature within 1° C.

TABLE I

CATALASE ACTIVITY OF PRESS-JUICE OF WINTER WHEAT LEAVES

Variety	Date 1923	Juice equiv. of diluted aliquot		Time in seconds to split 20 cc. increments of O_2 from 10 cc. Dioxygen					Total time for 100 cc.	Av. rate per gm. solid cc./gm./sec.
		Vol. cc.	Solids mg.	1st 20 cc.	2nd 20 cc.	3rd 20 cc.	4th 20 cc.	5th 20 cc.		
Seeding of July 16										
Minhardi	Sept. 6	1	140	61	37	23	27	33	181	3.95
Turkey	Sept. 6	1	125	78	48	—	—	—	—	(2.54)
Fulcaster	Sept. 6	1	100	99	51	—	—	—	—	(2.67)
Minhardi	Sept. 14	1	119	101	69	83	72	89	414	2.03
Turkey	Sept. 14	1	115	81	61	57	57	54	310	2.80
Fulcaster	Sept. 14	1	110	117	89	93	87	97	483	1.88
Seeding of Aug. 15										
Minhardi	Oct. 4	1	160	23	30	33	32	41	159	3.93
Turkey	Oct. 4	1	165	124	179	214	265	316	1098	0.55
Fulcaster	Oct. 4	1	120	170	145	230	240	361	1146	0.27
Minhardi	Oct. 25	$\frac{1}{3}$	92	33	35	37	37	38	180	6.04
Turkey	Oct. 25	$\frac{1}{3}$	86	40	43	50	56	57	246	4.73
Fulcaster	Oct. 25	$\frac{1}{3}$	64	84	86	85	89	105	449	3.48
Minhardi	Nov. 16	$\frac{1}{3}$	128	18	27	34	39	43	161	4.85
Turkey	Nov. 16	$\frac{1}{3}$	89	26	33	37	40	46	182	6.17
Fulcaster	Nov. 16	$\frac{1}{3}$	84	65	61	61	67	71	325	3.66
Minhardi	Dec. 14	$\frac{1}{3}$	128	24	38	48	54	62	226	3.46
Turkey	Dec. 14	$\frac{1}{3}$	132	46	86	79	109	129	449	1.69
Fulcaster	Dec. 14	$\frac{1}{3}$	140	46	80	86	71	95	378	1.89

Results

In addition to reporting the time readings directly, in Table I, the average rate for the whole volume of 100 cc. (or 40 cc. in two cases) expressed as cc. oxygen per gm. solids in juice per sec., is also given. The time readings are of course inversely proportional to the activity of the sample of juice. The wheat varieties are arranged in the table in the order of their winter hardiness, so that the relation between activity and hardiness may be seen at a glance.

With two exceptions (Turkey, Sept. 14, and Fulcaster, Dec. 14) the results show in all collections a direct correlation between the catalase activity per cc. juice and winter hardiness. With two further exceptions (Fulcaster, Sept. 6, and Turkey, Nov. 16) this is also true on the basis of activity per gm. solids in juice. As the season progressed, the activity of the juice increased very markedly, so much so that it was necessary to reduce the aliquots used from the equivalent of 1. cc. juice to $\frac{1}{3}$ cc., to keep the evolution of oxygen at a manageable rate. This is largely due to the increasing concentration of the juice resulting from the reduction in moisture content which characterizes the hardening process. Since this moisture-reduction process is as a rule more marked in hardy varieties (6, 7), and their catalase activity per gram solids in juice is also more pronounced, it follows that the catalase activity of their tissues must be generally greater than that of non-hardy varieties.

The catalase activity fluctuates quite violently in different collections, possibly reflecting the influence of changes in weather conditions on the physiological activity of the plants. It is a point of considerable interest that the activity appeared to reach a peak in the collections of Oct. 25 and Nov. 16, just the period when sugar accumulation was approaching the maximum (7).

Assuming that catalase and respiratory activity are related, the results obtained would suggest a greater rate of respiration in hardy varieties. On the other hand, hardy varieties generally maintain their sugar reserves during the winter better than non-hardy varieties. These two statements can scarcely be reconciled without investigating the low-temperature respiration rates of the varieties concerned, a subject to be discussed in the next paper of this series. In the meantime it should be noted that, while some workers have found a relation between catalase and respiration, this is not universally admitted, some holding that catalase is rather a protective agent against excessive oxidation. It may easily be imagined that a heavy accumulation of such a readily oxidizable substance as sugar would be accompanied by a protective mechanism of the kind suggested. It should be noted, too, that while the plants were collected from the field where the temperatures were low, at least during the latter part of the work, the catalase measurements were made at 20° C. It was assumed that the catalase in all samples of juice would have the same temperature coefficient, and that the relation of the varieties would not be changed by working at ordinary room temperature. While there seems no reason to doubt this assumption so far as press-juice is concerned, in the actual cells of the living tissues other factors may be operative to change the relations at low temperatures. It should also be noted that catalase activity in certain cases, for example in resting seeds, is taken as an expression of vitality rather than activity, of potential rather than kinetic energy. This may perhaps be the case in leaves of winter wheat.

While too much stress should not be laid on the results of a single series of experiments, it is important to note that this very simple determination appears to provide a rather sharp index of the relative hardiness of varieties of winter wheat.

References

1. Appleman, C. O. Relation of catalase and oxidases to respiration in plants. Maryland Agr. Exp. Sta. Bull. 191, 1915.
2. Burge, W. E. Why chloroform is a more powerful and dangerous anesthetic than ether. Science, 46: 618-620. 1917.
3. Harvey, R. B. Relation of catalase, oxidase, and H^+ concentration to the formation of overgrowths. Am. J. Botany, 7: 211-221. 1920.
4. Heinicke, A. J. Factors influencing catalase activity in apple-leaf tissue. New York (Cornell) Agr. Exp. Sta. Mem. 62. 1923.
5. Leggatt, C. W. Catalase activity as a measure of seed viability. Sci. Agr. 10: 73-110. 1929.
6. Newton, R. The nature and practical measurement of frost resistance in winter wheat. Univ. of Alta. Coll. of Agr., Res. Bull. 1, 1924.
7. Newton, R. and Brown, W. R. Seasonal changes in the composition of winter wheat plants, in relation to frost resistance. J. Agr. Sci. 16: 522-538. 1926.

RESPIRATION OF WINTER WHEAT PLANTS AT LOW TEMPERATURES[1]

By R. Newton[2] and J. A. Anderson[3]

Abstract

Respiration measurements at −7°, 0° and 7°C. with four winter wheat varieties and two spring wheat varieties grown in the field, were carried on during the fall and early winter of four years. The rate of respiration in winter wheat decreased as the plants hardened. At the outset all varieties respired about alike. After hardening began, the rates at −7°C. were in the inverse order of hardiness, with the spring wheats faster than the least hardy winter wheat. As hardening progressed, the differentiation of the winter varieties at −7° C. became sharper, and could be observed also at 0° C., but at 7° C. very little if any relationship of this sort developed. The spring varieties, because of frost damage, could not be compared with winter varieties in the fully hardened condition, but their behavior early in the season confirms the general conclusion that at the freezing point or lower temperatures respiratory activity is inversely related to winter hardiness. This accounts for the observed fact that hardy varieties maintain their sugar reserves better than non-hardy varieties during the winter.

Introduction

The respiration of wheat plants at low temperatures was investigated in order to explain if possible the disappearance of sugar during the winter. The naturally occurring sugar concentrations in winter varieties had been studied for a number of seasons, and the results had amply confirmed the finding of other investigators that sugar content is directly related to frost resistance. This field of work has been reviewed quite recently by Åkerman (1). Experiments in this laboratory (4, 6) had also indicated that the value of sugar lay in its ability to retard or prevent the precipitation and denaturation of the cell proteins by frost; or by acid or salt concentration, the two factors commonly supposed to account for frost precipitation.

Maximum concentrations of sugar in winter wheat plants at Edmonton are usually found about the end of November, after which they fall off gradually. Hardy varieties have in general a higher concentration and a slower rate of loss. This suggested that they might have a more profound dormancy, characterized by a slower rate of respiration, and possibly also a more stable dormancy, and be less easily wakened into a more frost-susceptible condition of physiological activity by occasional warm periods during the winter.

Govorov (2) published in 1923 a study of the differences in respiration of winter and spring cereals. The carbon dioxide respired was determined by a Petenkoffer apparatus, the plants being placed in a large flask immersed in a darkened water bath. He found that at 21° C., a hundred plants of a spring variety of each of wheat and rye gave off slightly more carbon dioxide than a

[1] *Manuscript received June 16, 1931.*
Contribution from the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. The authors wish to acknowledge also the technical assistance of A. W. Saddington in carrying out the measurements of 1928.

[2] *Professor of Field Crops and Plant Biochemistry, University of Alberta.*
[3] *Junior Research Biologist, National Research Laboratories of Canada.*

corresponding lot of a winter variety. When the temperature of the bath was reduced to 0° C. for 12 hr. and then raised to 5° C., the differences between the spring and winter forms were increased. In a further experiment in which it was raised only to 3° C., the differences were still greater. No exact parallelism was found between the rate of respiration and winter hardiness. Presumably the differences he found between winter and spring varieties might be expected to exist in a lesser degree between hardy and non-hardy varieties of winter wheat.

In 1927, there appeared a report by Martin (3) on the respiratory rates of one spring and three winter wheats, and one winter rye, grown in pots in the greenhouse. The soil around the plants was sealed with a half-and-half mixture of vaseline and liquid paraffin, the plants covered with a vessel sealed at the edges with the same mixture, and the pots transferred successively to chambers at 5°, 0°, −5° and −10° C. At each stage, 8 to 12 hr. was allowed for the plants to come to equilibrium, after which air was passed through and the carbon dioxide evolution measured. The spring wheat did not survive the lowest temperature. Triplicate determinations and averages of these are given for each variety at each temperature, together with the van't Hoff coefficient for each variety between −10° and 0° C. He concludes that at −10° C. the rate per unit dry matter in the winter wheat varieties is in the inverse order of hardiness, and that the temperature coefficients of the winter wheats and rye are in the order of hardiness. A study of his table shows that the variations between replicates are so wide as to cast doubt on the exactness of the method, and his conclusions seem barely justified by the data.

The investigations of low temperature respiration reported in this paper were begun in the fall of 1925 and continued in the falls of the three succeeding years, being completed in December, 1928. The results reported here are confined to those obtained in the latter part of the third season and in the fourth season. Prior to this time, the apparatus and procedure were being continually modified and refined to meet technical difficulties which appeared one after another, as it became more and more apparent that the differences between varieties were very small and would require a precision of measurement which had not at first been anticipated. It is considered that the more accurate results obtained with the final apparatus are sufficient to establish the relationship under investigation.

The temperatures used were 7°, 0° and −7° C. These were selected partly because they made a substantial range and also fell conveniently within the capacity of our equipment, and partly because −7° C. appeared to be a point of special physiological significance. It is the temperature at which absolute killing of unhardened greenhouse tissue occurs and the point of maximum frost precipitation of the press-juice proteins (4). A series of varieties of graded resistance to frost were used, to ascertain the relation between respiratory activity and hardiness.

Materials

One of the greatest limitations to precision in biological work is the plastic, almost fluid, nature of the experimental material. It was considered essential

that in order to make the results comparable with the behavior of wheats under normal winter conditions in the field, the respiration measurements should be made upon plants grown in the field and hardened naturally by exposure to fall frosts. This greatly increased the difficulties. Slight differences in environment in different parts of a field plot, or changes in conditions from day to day, or even hour to hour, are reflected in the physiological activity of the plants. Varietal differences in low-temperature respiration are small, and the natural variation between two groups of plants of the same variety may be as great as the average difference between two varieties. It was sought to overcome these errors by collecting plants at the same hour every day, by running determinations on all varieties at the same time, and by repeating the experiments often enough to produce reliable averages.

Another important difficulty arising out of growing the plants in the field is that of obtaining uninjured tissues. It is well known that bruising and cutting increase the rate of respiration. In the Edmonton climate, the plants begin to harden with the first frosts about the middle of September. By the middle of October they are approaching their winter equilibrium, but when November comes, a cold snap, in which the temperature drops to 0° F. or below, is liable to occur. Such conditions, while not killing the plants entirely, seriously impair their usefulness for respiration measurements, since in all but the hardiest varieties the majority of leaves are killed, and even in the hardy varieties all but a few of the youngest leaves are frozen back for an inch or two from the tips. If snow comes early, the plants may be protected from both rapid changes in physiological condition and from freezing back at the tips of the leaves, but in brushing off the snow it is difficult to avoid some injury to the plants, especially in cold weather when the leaves are brittle with frost, or if an earlier thaw has caused the snow to freeze to the leaves. Without special measures, therefore, the period during which reliable results may be obtained is generally limited to a few weeks between the time when the plants become relatively hardened and the first severe frosts.

In 1928, the last season of this work, it was proposed to grow the plants in the field in boxes, these to be removed before danger of severe frost to a greenhouse kept at 0° C. When it became clear that the controlled greenhouse then in course of erection would not be ready in time, the varieties were seeded in the field in the usual way, in rows 1 ft. apart. Boards, 6 in. wide, were placed on edge between every fourth row, so that light burlap (Hessian cloth) could be stretched over the plants as a protection against snow. This was done every night after Sept. 28. Each morning the covering was rolled back so that the plants were exposed to normal light conditions. It so happened that there was no appreciable snowfall that season during the period of the experiments, but the covering doubtless retarded the freezing back of the tips of the leaves in the non-hardy varieties.

In all experiments reported in this paper, field-grown plants were used, from which the roots had been removed, all dead or wilted leaves stripped, and dead leaf-tips cut off slightly above the boundary of the living tissue to avoid injuring the latter.

The removal of the roots seems justifiable, since the leaves are the organs which determine winter survival in wheat. Killing begins at the tips and extends gradually downwards. If all leaves are killed to the base, the plant fails to survive. In this respect the cereals differ from legumes such as clover and alfalfa. In the latter, the roots and the crown buds are the critical points, the fall-grown leaves always dying off in the winter in this climate.

Four varieties of winter wheat and two varieties of spring wheat were used in these experiments. The winter varieties were Minhardi, Turkey, Fulcaster and Squarehead's Master, named in descending order of winter hardiness as found in our own experimental plots and elsewhere. For the purpose of the present experiments, the first two may be classified roughly as hardy and the other two as non-hardy. The data for the survival of these varieties at Edmonton during the five years 1924-29 are given in Table I. The respiration experiments extended over the last four years of this period.

TABLE I

PERCENTAGE SURVIVAL OF WINTER VARIETIES AT EDMONTON

Variety	1924–25	1925–26	1926–27	1927–28	1928–29	Average
Minhardi	60	93	70	90	95	82
Turkey	63	83	30	90	95	72
Fulcaster	15	48	10	45	25	29
Squarehead's Master	5	—	0	2	5	3

The spring wheat varieties were Marquis and Red Bobs. These, of course, are non-hardy. They were included in these studies in order to ascertain if there were characteristic differences in the low-temperature respiration of spring and winter wheats and to provide additional non-hardy material.

Apparatus

Since the rates of respiration of plants are most readily compared by measuring the rate of evolution of carbon dioxide, the devising of an apparatus for this purpose was the first consideration. The small amount of carbon dioxide evolved and the unusual temperature conditions raised numerous technical difficulties. These have been overcome and it is believed that the apparatus and method here described are satisfactory for the investigation.

Constant low temperatures are maintained by carrying out the determination in a refrigerator cooled by means of a "Frigidaire" cooling system. By making small adjustments in the starting mechanism of the "Frigidaire" motor it has been possible to obtain temperatures constant to 0.5° C. within a range of seven degrees above and below 0° C.

Reference to the photograph (Fig. 1) will show that the apparatus is built on and in the refrigerator. A continuous current of air is blown through the respiration chambers by means of a small rotary fan pump. The air is first freed from carbon dioxide by passing through a washing tower. Four of these

are hooked up in parallel in order to increase the efficiency of washing by cutting down the rate of bubbling in each. The towers are of the same construction as those used to absorb the carbon dioxide produced by respiration. A glass tube of 60 cm. length and 2.5 cm. diameter, constricted at

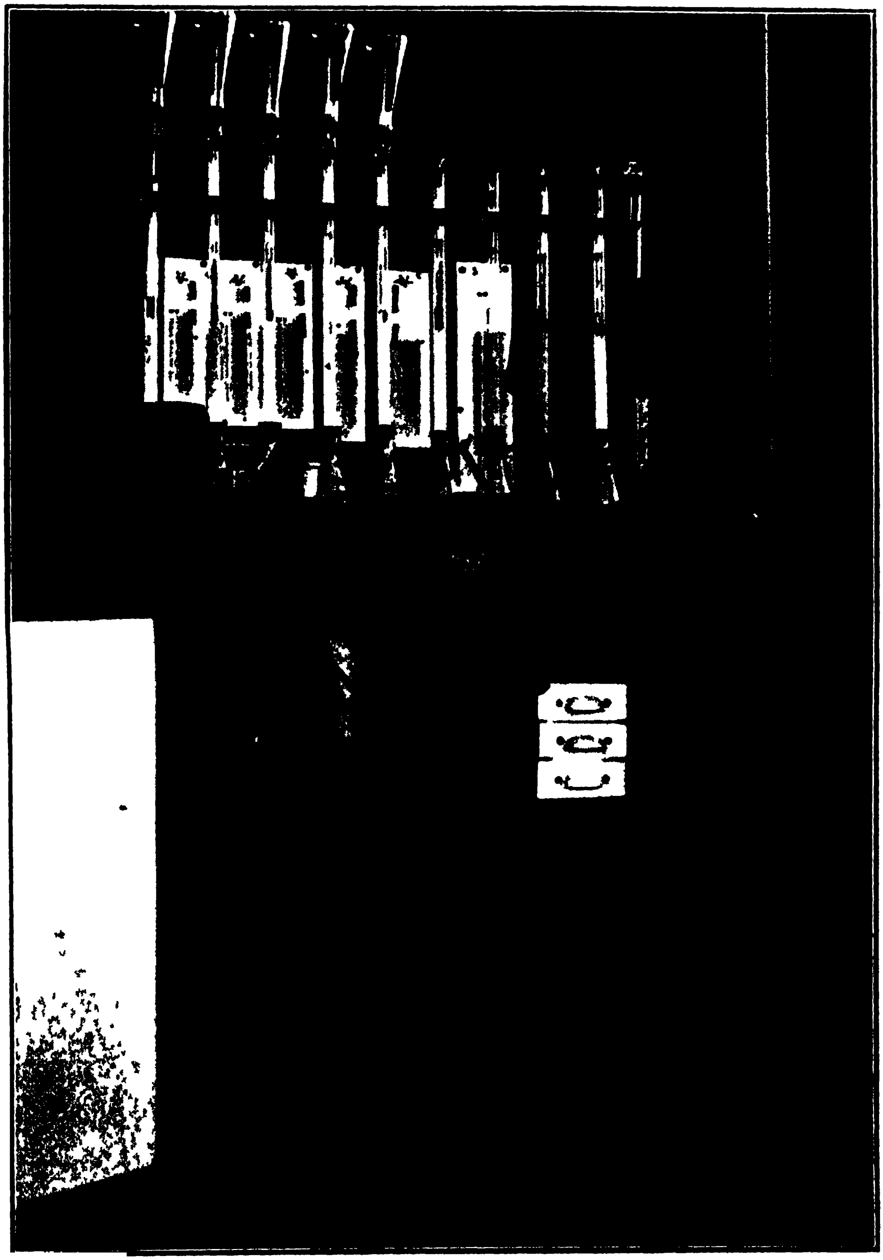


FIG. 1. *Apparatus used in low temperature respiration studies.*

the base so as to hold a perforated Gooch crucible plate, is tightly fitted through a rubber collar into a 300 cc. Erlenmeyer side-necked flask, the tube touching the bottom. The tube is filled to a height of 35 cm. with perforated glass beads, and 50 cc. of 20% sodium hydroxide solution is run in. A rubber stopper at the top of the tower carries the outlet tube. The lead from the washing towers enters a small bottle which acts as a trap for any sodium hydroxide which may froth over. The air is then conducted by means of a thick-walled rubber tube through a small hole in the refrigerator and into a large vertical brass tube, 70 cm. long and 2.5 cm. in diameter, the base of which is fitted into a small bottle by means of a rubber stopper. This tube collects the moisture deposited by the air on entering the refrigerator. The large diameter and upright position prevent stoppage of the air passage by the ice collected, which has been found to occur if the smaller tubes of the cooling system are connected directly with the washing towers. The air is next passed through 20 ft. of copper tubing, the coils of which are situated close to the cooling apparatus. It is then split into five currents, one entering the top of each of five respiration chambers. A thick-walled rubber tube conducts the air from the bottom of each respiration chamber through the top of the refrigerator to each of five absorption towers containing 50 cc. of 0.3 N sodium hydroxide solution. The rates of circulation of air through the towers are measured by capillary flow-meters. The air from each tower passes into a 500 cc. Erlenmeyer flask, which acts as an air-cushion and steadies the pressure, then through the flow-meter and out of the apparatus. The rate of flow through each respiration chamber is regulated to 15 litres per hour by means of a stopcock in the tube connecting the top of the tower and the flask.

The respiration chambers are galvanized iron cylinders of 3½ litres capacity, closed at the top and with inlet and outlet tubes at the top and bottom respectively. They are sealed by means of ordinary plasticine into circular grooves, ½ in. deep by ½ in. wide, in a wooden block impregnated with wax.

A fan keeps the temperature uniform throughout the refrigerator, and temperature records are kept by means of a recording thermometer and an alcohol thermometer passed through the wall of the refrigerator so that it can be read without opening the door.

Chief among the difficulties encountered in developing apparatus suitable for the measurement of very slow rates of respiration was that of washing the intake air so free of carbon dioxide that the blank might not be disproportionately large. This was effected as described by the use of four bead washing towers containing 20% sodium hydroxide solution and hooked up in parallel. The use of a washing system of such high resistance was made possible by a pumping rather than a suction system of air circulation. After the air had passed through the washing towers, the residual positive pressure in the respiration chambers was only about 3 cm. of mercury. This had the further advantage of making it impossible for any air to leak into the apparatus. The air-washing system is not perfect but was the most practical and efficient of a number which were tried. Blank runs always showed a small amount of carbon dioxide. This, however, was fairly constant and introduced no

appreciable error. In Tables II and III are given the blank determinations for all the runs in 1927 and 1928, of which the respiration results are recorded in Tables V to X. If reference is made to the latter tables, it must be remembered that the respiration rates are expressed as mg. carbon dioxide per gm. dry matter in the plants. The blanks were subtracted from the total carbon dioxide produced by all the plants in a given chamber, and were not excessive in relation to the whole amount so produced.

TABLE II

BLANK DETERMINATIONS, 1927

Date	CO_2 mg.	Date	CO_2 mg.	Date	CO_2 mg.	Date	CO_2 mg.
Oct. 23	2.6	Oct. 26	2.3	Oct. 24	3.6	Nov. 1	2.4
Oct. 23	2.7	Oct. 26	2.5	Oct. 25	2.7	Nov. 3	2.6
Oct. 23	2.9	Oct. 26	2.4	Oct. 27	2.5	Nov. 4	2.5
Oct. 23	2.9	Oct. 26	2.7	Oct. 28	2.2	Nov. 10	2.4
Oct. 23	2.6	Oct. 26	2.5	Oct. 31	2.5	Nov. 11	2.5

TABLE III

BLANK DETERMINATIONS, 1928

Date	CO_2 mg.	Date	CO_2 mg.	Date	CO_2 mg.	Date	CO_2 mg.
Sept. 10	3.2	Sept. 27	2.4	Oct. 17	4.2	Nov. 5	2.1*
Sept. 11	4.0	Sept. 28	1.7*	Oct. 18	3.4*	Nov. 6	3.8
Sept. 12	3.3*	Sept. 29	2.0	Oct. 19	3.6	Nov. 7	4.5
Sept. 13	2.3	Oct. 1	2.2	Oct. 22	4.1	Nov. 8	4.8
Sept. 14	2.8*	Oct. 2	3.8	Oct. 23	4.8	Nov. 9	3.2*
Sept. 15	2.4	Oct. 3	4.9	Oct. 24	2.4*	Nov. 10	4.7
Sept. 16	2.4	Oct. 8	2.9*	Oct. 25	2.2	Nov. 17	5.1*
Sept. 17	2.7	Oct. 9	3.1	Oct. 26	5.3	Nov. 20	5.0
Sept. 18	2.6	Oct. 10	3.5	Oct. 29	4.3	Nov. 21	0.5*
Sept. 19	4.2	Oct. 11	4.2	Oct. 30	4.3	Nov. 23	1.7
Sept. 20	2.7*	Oct. 12	3.5*	Oct. 31	2.5*	Nov. 27	1.7
Sept. 24	2.4	Oct. 13	3.5	Nov. 1	2.2	Nov. 28	2.4
Sept. 25	1.5*	Oct. 15	3.5	Nov. 2	1.9	Nov. 30	3.8
Sept. 26	1.9	Oct. 16	4.2	Nov. 3	4.2		

**Air-washing towers filled with fresh 20% solution of NaOH.*

An interesting point was noticed in the efficiency history of the washing towers in 1928. The figures marked with an asterisk in Table III represent the blanks found in the first run after changing the absorbing solution. In twelve out of fourteen cases, the fresh solution reduced the size of the blank. In one case six runs were made before changing the solution; in all others, five or less. The apparent falling off in absorbing efficiency is not easily explained, since calculation shows that the reduction in concentration of the solution is comparatively slight, but it indicates the importance of frequent changes.

Before adopting bead towers for absorbing the carbon dioxide produced in respiration, they were tested in comparison with Reiset towers. The former

are equally as efficient as the latter and have these advantages: that they required only one third as much reagent, an advantage in titration; that they can be much more easily cleaned, and that they are much cheaper. Sodium hydroxide is used in preference to barium hydroxide as the absence of precipitate makes the rinsing of the towers more efficient, and because it allows a simpler method of titration.

The foregoing description is of the final apparatus used in obtaining all results reported in this paper. Four chambers were used for plants in each series and the fifth remained empty for the blank determination.

Procedure

Before going out to the field to make collections the apparatus is hooked up completely, except for the respiration chambers, by joining the five-way joint to the tubes leading to the absorption towers. Fifty cc. of water are run into each tower and after starting the pump the rates of bubbling are adjusted by means of the stopcocks at the top. This preliminary run clears the apparatus of residual carbon dioxide. The windows and the outside door of the room are opened in order to allow it to reach a temperature as near that of the open as possible.

In making collections, loose snow, if present, is first carefully brushed off, and then if the ground is frozen a small axe is used to cut out lumps of earth containing the plants. The plants are brought back to the laboratory and the work of preparing them for the respiration chambers is carried out on a table outside the building. Dead or wilted leaves are stripped and dead leaf-tips cut off. The earth is carefully broken away from each plant and the roots are cut off just below the crown which is wrapped in a small ball of plasticine by means of which the plant is fastened to the base of the respiration chamber. About twelve to twenty-five plants, the number depending on their size, are thus grouped together. The covering chamber is then firmly pressed into the plasticine seal. The fan is stopped and the respiration chambers are carried in and hooked up as quickly as possible. The refrigerator is open about three minutes during this operation and the temperature rises slightly. After starting the fan and pump and readjusting the rates of bubbling, the temperature is brought down to normal by running the refrigerating machine continuously.

The apparatus is now given a further run of 2 hr. in order to bring the concentration of carbon dioxide throughout the system to equilibrium. The pump is then turned off and the absorption towers are cut off from the respiration chambers by means of screw clamps. The towers are disconnected and the water is run out through the side-neck of the flask. They are replaced and 50 cc. of 0.3 N sodium hydroxide is run into each from a standard pipette suitably protected from contamination by the carbon dioxide of the air. The actual determination of carbon dioxide evolved is now made by running the apparatus for 4 hr. The pump is then turned off and the stopcocks at the top and the screw clamps at the bottom of the towers are closed.

The titration is carried out in the original flask. The tube is drawn up from the bottom of the flask, washed down with eight small quantities of carbon-dioxide-free water, and the volume is then made up to 200 cc. Two drops of phenolphthalein are added, and the solution is titrated with approximately 0.3 *N* hydrochloric acid until the color begins to pale. The titration is then continued with 0.05 *N* standard hydrochloric acid until the colorless end-point is reached. Three drops of the British Drug House Universal Indicator are added and the titration continued until a slight change in the quality of the color gives the first true pink. The amount of carbon dioxide absorbed is calculated from the amount of acid required to titrate from the first, or bicarbonate end-point, to the final carbonate end-point. The above technique was adopted after experimenting with various combinations and quantities of different indicators, as being the most accurate and convenient for the determination of the amounts of carbonate usually present. Tests carried out with known solutions of sodium bicarbonate gave a recovery of 100% with a maximum error of 1%.

Experiments of 1927

The results prior to Oct. 24, 1927, are not reported, because, as already mentioned, the apparatus and procedure were being continually improved and it is felt that after these had taken final form a sufficient body of reliable data was obtained. The earlier data, though less precise than those secured later, support the same general conclusions.

The results of the respiration experiments extending from Oct. 24 to Nov. 11, 1927, are given in Tables V and VI, reporting respectively, a series with Minhardi to check the error of sampling, and a series with the four winter varieties.

In Table IV are given the minimum and maximum daily temperatures during and shortly before the period of the 1927 experiments. The columns here and in other tables of temperature are given in the reverse order to that usually

TABLE IV

TEMPERATURE RECORDS (°F.) DURING PERIOD OF RESPIRATION EXPERIMENTS, 1927

Date	Min.	Max.	Date	Min.	Max.	Date	Min.	Max.	Date	Min.	Max.
Oct. 19	34	55	Oct. 26	24	50	Nov. 2	12	51	Nov. 9	10	20
20	27	49	27	18	44	3	33	42	10	5	9
21	26	57	28	31	47	4	21	40	11	−7	8
22	29	55	29	31	48	5	13	39	12	−14	11
23	27	56	30	15	41	6	17	21	13	−14	6
24	23	51	31	18	33	7	10	19	14	−14	7
25	26	51	Nov. 1	12	37	8	10	26	15	−4	9

found in meteorological records, to have the readings in chronological order; *i.e.*, the minimum temperature for a given day usually occurs early in the morning.

After the apparatus had been brought to a state of maximum efficiency, the first experiment was designed to test the error of sampling. In Table V are given the results of three runs with Minhardi, made from Oct. 24 to 27, a period of very even temperatures (Table IV) except for a colder dip on the morning of the 27th, which may perhaps account for the slightly lower average rate of respiration on that day. For any one run, however, it seems legitimate to attribute the variations between replicates chiefly to sampling errors. Twelve plants were used in each chamber, and while an effort was made to select only plants which appeared representative, it is not surprising to find this small number insufficient to get rid of the natural variability of such material. This emphasized the importance of making as many runs as possible on which to base comparisons of varieties.

TABLE V

RESPIRATION MEASUREMENTS OF REPLICATE SAMPLES OF MINHARDI WHEAT AT 0° C.

Sample No.*	Mg. CO_2 per gm. dry matter per 4 hr. run			
	Oct. 24	Oct. 25	Oct. 27	Average
1.	1.67	1.67	1.77	1.70
2.	1.82	1.95	1.65	1.81
3.	1.96	1.96	1.75	1.89
4.	1.88	1.83	1.63	1.78
Average	1.83	1.85	1.70	1.79

**Samples collected at 11 a.m.*

From October 6 to October 24, when the foregoing special experiment with Minhardi was done, the weather had become gradually colder. On October 7 the temperature fell to 18° F. This killed back the tips of the leaves of the two spring varieties to such an extent that they were useless for further work. The series of runs reported in Table VI was thus restricted to the winter varieties,

TABLE VI

RESPIRATION MEASUREMENTS OF WINTER WHEATS AT −7°, 0° AND 7° C., OCT. 28–NOV. 11, 1927

Variety*	Mg. carbon dioxide per gm. dry matter per 4 hr. run									
	−°7 C.			0° C.				°7 C.		
	Nov.4	Nov.10	Av.	Oct.28	Oct. 31	Nov.1	Av.	Nov.3	Nov.11	Av.
Minhardi	0.56	0.46	0.51	1.58	1.76	1.78	1.77	2.96	4.44	3.70
Turkey	0.49	0.42	0.46	1.66	1.60	1.68	1.65	2.60	3.77	3.19
Fulcaster	0.86	0.50	0.68	1.45	1.67	1.88	1.67	3.07	4.78	3.93
Sq. Master	0.86	0.53	0.70	1.63	1.88	2.09	1.87	3.30	3.68	3.49

**Samples collected at 11 a.m.*

which by now had been hardened by exposure to fairly severe frosts. The plants were still comparatively undamaged, although the oldest leaves had to be removed, because brown and dead, and the tips of some of the other leaves cut off, because killed by frost. The cold snap beginning on the night of Nov. 9-10, however, killed a considerable number of the leaves of Fulcaster and Squarehead's Master, and the results obtained with these varieties on Nov. 10 and 11 must therefore be accepted with reserve.

It will be noted that in Table VI and all other tables of respiration results, the varieties are arranged in order of winter hardiness.

Considering all the results in Table VI, it appears that Fulcaster and Squarehead's Master, the two non-hardy varieties, respired more rapidly than the others at $-7°$ C., that Squarehead's Master had the greatest rate at 0° C., and that at 7° C. there were no definite differences. If we eliminate the runs of Nov. 10 and 11, when Fulcaster and Squarehead's Master had been injured by frost, the differences at $-7°$ C. are increased; these two varieties also respired slightly more rapidly than the others at 7° C. Bearing in mind the variation between replicates, not much weight should be given to the differences found at temperatures other than $-7°$ C., but the results on the whole are in agreement with those found earlier in the same season as well as in the previous seasons.

On November 14 all varieties were collected, thawed out gradually and examined. Almost all the leaves of Squarehead's Master were dark green and watery looking. Fulcaster was not much better and 80% of the leaves of Turkey were in a similar condition. In Minhardi only the tips of the leaves

TABLE VII

TEMPERATURE RECORDS (°F.) DURING PERIOD OF RESPIRATION EXPERIMENTS, 1928

Date	Min.	Max.	Date	Min.	Max.	Date	Min.	Max.	Date	Min.	Max.
Sept.			Sept.			Oct.			Nov.		
8	28	64	29	42	80	20	23	45	10	22	42
9	32	71	30	39	52	21	18	56	11	17	46
10	33	75	Oct.			22	30	55	12	23	53
11	37	71	1	33	54	23	26	50	13	15	38
12	40	73	2	36	43	24	17	52	14	15	33
13	37	75	3	28	40	25	25	56	15	8	37
14	47	65	4	17	41	26	28	37	16	8	37
15	37	60	5	22	49	27	18	33	17	17	37
16	27	69	6	25	61	28	17	40	18	5	37
17	35	80	7	30	56	29	13	40	19	13	43
18	37	67	8	32	52	30	11	33	20	14	43
19	34	53	9	29	52	31	8	29	21	25	42
20	38	62	10	33	38	Nov.			22	19	52
21	44	65	11	21	39	1	8	42	23	20	38
22	43	57	12	20	44	2	9	55	24	10	42
23	41	55	13	23	59	3	10	58	25	14	43
24	25	57	14	28	58	4	14	52	26	9	34
25	21	55	15	24	55	5	20	48	27	10	22
26	18	62	16	26	59	6	20	50	28	10	37
27	22	65	17	35	40	7	18	48	29	10	38
28	32	80	18	25	55	8	16	49	30	21	39
			19	29	52	9	18	52			

were damaged. The plants were afterwards put in water and kept at about 12° C. The dark green leaves failed to become turgid and proved to be dead. Under these conditions it was considered useless to continue the investigation. The work of 1927 and of the preceding two years, however, besides providing useful indications of the relations between varieties, resulted we believe in the development of the maximum efficiency possible in measuring respiration rates with the type of apparatus used. More reliable results can only be obtained by growing the plants under controlled conditions.

Experiments of 1928

It has already been stated that the field plots in 1928 were covered with burlap every night after Sept. 28, to protect the plants from snow and also to some extent from frost-killing of the leaf-tips. The object was to prolong the period during which respiration measurements could be made with uninjured plants. In order to lengthen the season further by starting the experiments earlier, two seedings were made of all varieties, the first on Aug. 3 and the second on Aug. 20. The latter is the normal date for seeding winter wheat at Edmonton. The temperature records for the period of the experiments are given in Table VII. The plants for all experiments this season were collected at about 10 a.m. on the dates specified in the tables.

By the time the respiration determinations were started on September 10 the spring wheat plants of the first seeding (Aug. 3) had grown to a height of 12 in. The respiration chambers used were only 7½ in. high so that although the number of plants per run was reduced, they had to be crowded and perhaps slightly crushed in order to get them into the chambers. The winter wheats, of course, had not made so much growth, the average height of the plants being about 6 in. These plants were quite satisfactory in size.

After September 20, plants seeded on August 20 were used. These were within the limitations of size imposed by the design of the respiration chambers.

From September 24 to 27 there were heavy frosts each night. Considerable damage was done to the spring wheats but none to the winter varieties. By September 29 half the leaves of Marquis and Red Bobs had to be stripped entirely and the tips of many of the remaining leaves cut back, so that only sound tissue might be used for the determinations. Up to the 10th of October the damage to the spring wheats became progressively worse. By that date only one or two green leaves remained on each plant and the work with these varieties was discontinued. During this period the winter wheats remained in good condition.

By October 17 Squarehead's Master began to show injury at the leaf tips and by the 25th many of the leaves had to be removed entirely and most of the rest showed tip injury. During the nights of October 29-31 heavy frosts touched the leaf-tips of Turkey and Fulcaster. The Minhardi plants still remained normal.

No further heavy frosts came until the week of Nov. 10-17. After these frosts the Minhardi showed injury to the leaf tips. The three hardier varieties (Minhardi, Turkey and Fulcaster) all showed considerable wilting, a result of

prolonged exposure to dry, cold weather. The wilted leaves were stripped before the experiments. The plants of Squarehead's Master had only one or two leaves apiece in suitable condition for the tests. The experiments were discontinued on November 30.

TABLE VIII

RESPIRATION MEASUREMENTS OF WINTER AND SPRING WHEATS AT −7°, 0° AND 7° C., 1928

Mg. carbon dioxide per gm. dry matter per 4 hr. run

Variety	−7°C.				0°C.				7°C.		
(a)	Sept. 17	Sept. 18	Sept. 19	Sept. 20	Sept. 10	Sept. 11	Sept. 12	Sept. 16	Sept. 13	Sept. 14	Sept. 15
Minhardi	1.50	1.26	—	1.05	2.15	—	2.26	—	4.86	—	3.10
Turkey	—	—	0.92	1.23	—	1.88	2.08	2.02	—	4.71	5.12
Fulcaster	—	1.34	1.25	—	2.08	—	—	1.56	4.18	4.19	—
Sq. Master	1.85	1.63	—	—	2.44	1.97	—	2.13	5.14	5.25	—
Red Bobs	—	—	1.38	—	—	1.98	1.93	—	—	4.07	4.55
Marquis	1.54	—	—	1.37	2.56	—	2.11	1.74	4.09	—	4.64

Variety	−7°C.						0°C.			7°C.		
(b)	Oct. 1	Oct. 2	Oct. 3	Oct. 8	Oct. 9	Oct. 10	Sept. 24	Sept. 25	Sept. 26	Sept. 27	Sept. 28	Sept. 29
Minhardi	0.86	—	—	0.76	1.06	—	2.29	—	2.38	4.30	—	4.55
Turkey	0.98	—	0.81	1.05	1.27	—	—	2.18	2.40	—	4.25	4.24
Fulcaster	—	1.14	0.85	1.14	—	1.22	1.73	2.17	—	4.90	4.41	—
Sq. Master	—	1.18	0.87	0.91	—	1.52	2.62	2.66	—	4.47	3.68	—
Red Bobs	1.08	—	0.79	—	1.53	1.69	—	2.61	2.37	—	3.97	4.04
Marquis	1.27	1.63	—	—	1.82	1.85	2.24	—	2.36	3.98	—	5.03

Variety	−7°C.					0°C.					7°C.			
(c)	Oct. 12	Oct. 16	Oct. 19	Oct. 24	Oct. 26	Oct. 11	Oct. 15	Oct. 18	Oct. 23	Oct. 29	Oct. 13	Oct. 17	Oct. 22	Oct. 25
Minhardi	0.67	0.78	0.23	0.89	0.70	1.69	1.52	1.59	1.34	1.63	4.25	2.98	2.96	2.96
Turkey	0.71	0.91	0.49	0.87	0.75	1.45	1.60	1.64	1.19	1.76	4.38	3.02	3.20	3.35
Fulcaster	0.90	1.09	0.84	0.93	0.83	1.64	1.20	1.41	1.55	1.87	4.45	3.04	3.00	3.05
Sq. Master	1.07	1.14	0.93	1.20	0.80	1.98	1.53	1.94	1.76	2.39	5.77	4.64	3.87	3.70

Variety	−7°C.				0°C.			7°C.			
(d)	Oct. 31	Nov. 2	Nov. 7	Nov. 9	Nov. 1	Nov. 5	Nov. 8	Oct. 30	Nov. 3	Nov. 6	Nov. 10
Minhardi	0.35	0.78	0.70	0.61	1.20	1.47	1.18	3.33	2.71	2.51	2.39
Turkey	0.54	—	0.65	0.73	1.43	1.53	0.94	3.31	2.96	2.21	2.70
Fulcaster	0.60	1.12	0.88	0.89	1.45	1.37	1.32	3.78	3.06	2.55	2.59
Sq. Master	0.81	1.23	0.81	0.85	1.31	1.77	1.83	4.49	3.77	2.35	3.58

Variety	−7°C.		0°C.			7°C.	
(e)	Nov. 21	Nov. 30	Nov. 17	Nov. 20	Nov. 27	Nov. 23	Nov. 28
Minhardi	0.43	0.60	1.07	1.28	0.94	2.44	2.64
Turkey	0.72	0.39	1.30	1.16	1.04	1.90	2.90
Fulcaster	0.86	0.36	--	1.43	1.07	2.88	2.17
Sq. Master	0.67	0.55	—	1.66	1.60	3.11	2.62

The results of the 55 runs made in 1928 are in Table VIII divided into five sections, corresponding to the periods described above, *viz.*,: (a) while plants from the first seeding were used; (b) while it was possible to continue the experiments with both winter and spring wheats from the second seeding; (c) after the spring wheats had been discontinued because of frost damage, and while all the winter wheats but Squarehead's Master remained untouched by frost; (d) after the leaf-tips of Turkey and Fulcaster had been touched; (e) after Minhardi leaf-tips showed injury. Since only four varieties could be included in one run, a rotation was necessary when both spring and winter varieties were used. Accidents sometimes reduced the number of records obtained to three, especially in the first section of the table. After the spring varieties were discontinued, all the others were included in every run, and the three experimental temperatures were used in rotation, generally on successive days.

The results show considerable variation in the respiration of samples of the same variety at the same temperature on different days. No doubt this is due partly to the natural variability of the plants, an unavoidable source of error already noted, and partly to changes in weather conditions. The general effect of temperature is clearly seen in the gradual reduction of respiration rates as the season advances. To minimize the effect of sampling errors and temperature fluctuations, the data have been summarized in Table IX by periods corresponding to the sections of Table VIII. Each figure is the average rate found in all the runs of a given period.

TABLE IX

SUMMARY OF RESPIRATION MEASUREMENTS, 1928

Variety	Mg. CO_2 per gm. dry matter per 4-hr. run			Variety	Mg. CO_2 per gm. dry matter per 4-hr. run		
	−7°C.	0°C.	7°C.		−7°C.	0°C.	7°C.
(a) Sept. 10–20				(c) Oct. 11–29			
Minhardi	1.27	2.20	3.98	Minhardi	0.65	1.59	3.19
Turkey	1.08	1.99	4.92	Turkey	0.75	1.52	3.49
Fulcaster	1.30	1.82	4.18	Fulcaster	0.92	1.53	3.38
Sq. Master	1.74	2.18	5.20	Sq. Master	1.03	1.92	4.50
Red Bobs	1.38	1.96	4.31	(d) Oct. 30–Nov. 10			
Marquis	1.46	2.14	4.36	Minhardi	0.61	1.28	2.74
				Turkey	0.64	1.27	2.80
(b) Sept. 24–Oct. 10				Fulcaster	0.87	1.35	3.00
Minhardi	0.89	2.34	4.42	Sq. Master	0.92	1.64	3.55
Turkey	1.03	2.29	4.24	(e) Nov. 17–30			
Fulcaster	1.09	1.95	4.66	Minhardi	0.51	1.10	2.54
Sq. Master	1.12	2.64	4.08	Turkey	0.55	1.17	2.40
Red Bobs	1.27	2.49	4.00	Fulcaster	0.61	1.25	2.52
Marquis	1.64	2.30	4.50	Sq. Master	0.61	1.63	2.86

As already noted, the spring wheat plants used during the first period were seeded on Aug. 3, and were so large they had to be crowded into the respiration chambers. This crowding may on the one hand have restricted the free circulation of air and so cut down the rate of respiration, while on the other hand

enough tissue may have been crushed to accelerate the rate of evolution of carbon dioxide. No net effect of the crowding is shown by the results, which at 0° and 7° C. were not significantly different from those obtained with winter wheat plants of smaller size. As all varieties were during this period in an unhardened condition, differences related to winter hardiness would scarcely be expected. There is, however, an indication that the spring wheats and Squarehead's Master, an English winter wheat definitely non-hardy at Edmonton, respired slightly faster than the others at −7° C.

The change on September 24 to the smaller plants seeded on August 20 coincided with the incidence of heavier frosts. The period therefore includes all determinations made with spring wheat plants from which gradually increasing amounts of tissue had to be discarded because of frost damage, and winter wheats in a partly hardened but undamaged condition. Here some additional evidence of differentiation appears in the respiration rates at −7° C., which fall in the inverse order of hardiness of the winter wheats, with the spring wheats definitely faster than the least hardy winter variety. At 0° and 7° C., no differences were demonstrated.

The remaining three periods of experiments with winter wheats are characterized by gradually decreasing rates of respiration and a sharper differentiation on the basis of hardiness, which now becomes evident at 0° C. though still more distinct at −7° C.

The results of the entire series of 1928 experiments will now be analyzed in greater detail, with special reference to the winter varieties.

Measurements at −7° C. In Table VIII it will be seen that there were ten runs at −7° C. in which all four winter varieties were compared, and eleven additional runs in which two or three of them were included. In fourteen of these twenty-one runs the values for all varieties fell in the inverse order of hardiness. In Table X are shown the results obtained in the remaining seven runs. It will be seen that in four out of the seven runs the two hardy varieties fall in one group characterized by slower respiration than the other group of two non-hardy varieties. The difference in hardiness between Minhardi and Turkey, or between Fulcaster and Squarehead's Master, is not as great as the difference between the pairs. The latter difference is so great that the application of the terms hardy and non-hardy respectively is amply justified. The division of the results into two groups is therefore almost as satisfactory as if they had come in the expected order for individual varieties. Two of the three series of measurements given in Table X which show no regularity are the last two of the season and were made at a time when the plants of all varieties were showing considerable frost injury. Including all runs, however, there are out of the twenty-one just three which do not support the conclusion that hardy varieties have a lower rate of respiration than non-hardy varieties at −7° C.

Measurements at 0° C. The summaries of the measurements made at 0° C. (Table IX) show no regularity until the last two periods. From Oct. 30 to Nov. 10, Minhardi and Turkey had the same average rate of respiration, and the two non-hardy varieties higher rates, the least hardy being highest. In the last period the rate of respiration was in all varieties inversely proportional to

TABLE X

RESULTS OF THOSE MEASUREMENTS AT −7°C. WHICH DID NOT FALL IN INVERSE ORDER OF HARDINESS FOR ALL VARIETIES

Date 1928	Mg. carbon dioxide per gm. dry matter per 4 hr. run			
	Hardy varieties		Non-hardy varieties	
	Minhardi	Turkey	Fulcaster	Sq. Master
Oct. 8	0.76	1.05	1.14	0.91
Oct. 24*	0.89	0.87	0.93	1.20
Oct. 26*	0.70	0.75	0.83	0.80
Nov. 7*	0.70	0.65	0.88	0.81
Nov. 9*	0.61	0.73	0.89	0.85
Nov. 21	0.43	0.72	0.86	0.67
Nov. 30	0.60	0.39	0.36	0.55

**In these series the pairs of hardy and non-hardy varieties did fall in inverse order of hardiness.*

hardiness, as at −7° C. A similar condition is of course shown by the data for the individual runs in Table VIII. These show no regularity until October 23. Of the seven runs made after that date, the results of three fall in the expected order for all varieties, three divide the varieties into the hardy and non-hardy groups, and in the one remaining run Fulcaster had the lowest rate of respiration. This is apparently fairly typical of the behavior of this variety at this temperature. In the second and third periods the results for Fulcaster are lower than its hardiness would lead one to expect.

Measurements at 7° C. The measurements at 7° C. show little definite trend at any stage of the experiment. Only in three of the sixteen runs did the results for all four varieties fall in the inverse order of winter hardiness, though in a few other cases they fell into the hardy and non-hardy pairs. Whether these cases are fortuitous or significant it is difficult to say.

Discussion

The results presented after four seasons study of the respiration of wheat plants at low temperature are considered to establish definitely that at −7° C. the rate in winter varieties is in the inverse order of hardiness. The differentiation is indefinite early in the season, but becomes increasingly sharp as the plants advance into the fully hardened condition. At 0° C. the same differences could be observed, in a less marked degree, after the plants had become hardened. At 7° C. the relationship practically disappeared.

During the time in which it was possible to work with the spring wheats, they showed, as compared with the winter varieties, a higher rate of respiration at −7° C. Their susceptibility to frost made it impracticable to determine whether this differentiation would extend to the rate at 0° C. in the hardened condition. The results as they stand may be regarded as confirming and extending the conclusion that at −7° C. the rate of respiration in wheat plants is inversely proportional to winter hardiness.

The significance of this conclusion lies in the fact that in this climate winter plants are for long periods covered with snow at temperatures below the freezing point. Under these conditions the hardier varieties of wheat would appear to conserve more effectively their sugar reserves, which are an important protection against frost denaturation of the cell proteins. Furthermore, the slower respiration at freezing temperatures is an expression of the plant's ability to go more rapidly and completely into a dormant condition in which sensitivity to frost injury is reduced. The latter change is probably connected with the colloidal condition of the cell contents.

The decrease in rate of respiration as the season progresses is probably a temperature effect and one manifestation of the hardening process. All varieties started from approximately the same level, but the reduction in rate was carried farther in the hardy varieties.

Earlier investigations in this laboratory have shown the leaf blades to be considerably richer in sugar than the leaf sheaths. It might therefore be suggested that the cutting-back or removal of leaf blades in the later periods of the respiration experiments would reduce the net concentration of sugar in the tissues and so depress the rate of respiration. To answer this, it may be pointed out that sugar concentration is evidently not the limiting factor, since hardy varieties contain more sugar and yet respire more slowly. Moreover, Minhardi, of which the leaves remained untouched until the last period in 1928, fell off in respiration rate more markedly than any of the others. On the other hand, it may be argued that the cutting and bruising of the tissues incident to the removal of blades and tips would be expected to accelerate respiratory reactions. Following the heavy frosts of the nights of October 29-31, 1928, which touched the leaf-tips of Turkey and Fulcaster, the difference between the rates of respiration of these varieties and Minhardi, which remained untouched, were accentuated. Against this, however, must be set the observation of a similar differentiation between the winter varieties after the first hardening frosts of October and before any injury to the tissues had taken place. On the whole, therefore, it seems legitimate to regard the changes as an expression of the relative adaptations of the varieties to winter resistance.

A comparison of the daily variation in respiratory rates with the daily fluctuations in outdoor temperature suggests that there is a considerable lag in the response of physiological state to temperature. The comparative stability of the progressive change in state of the plants brought about by the hardening process is clearly shown in the respiration rates found, which decreased throughout the season, notwithstanding that all measurements were made at the same series of temperatures. On the other hand, physiological activity, within the limits of the state of dormancy at any given time, evidently responds very rapidly to temperature, since only 2 hr. were allowed for plants to come to equilibrium in the refrigerator before making the measurement, and at 7° C. all varieties respired at about the same rate, whereas at $-7°$ C. they were sharply differentiated.

Of the two suggestions put forward at the outset of this work, to account for the slower rate of sugar loss from hardy varieties, only the first, that they might have a more profound dormancy characterized by a slower rate of respiration at winter temperatures, has been proved by these experiments. The other suggestion, that they might have a more stable dormancy and be less easily wakened into a more frost-susceptible condition of physiological activity by occasional warm periods during the winter, seems not to be supported by the behavior of the varieties at 7° C. It has been noted, however, that these were only small changes in activity within the limits of the state of dormancy at the time. The possible effect on this state of a thaw lasting some days has still to be investigated.

In passing, it may be noted that catalase activity, which is believed by some investigators to be directly related to respiratory activity, was found to be directly related to winter hardiness in wheat (5). Though the leaves of hardened plants were used, the catalase determinations on the press-juice were carried out at 20° C. The present experiments do not make it possible to say whether the respiratory activity of hardened plants at this temperature would be in the order of hardiness. It is clear, however, that catalase activity as ordinarily determined is inversely related to low-temperature respiration.

References

1. Åkerman, Å. Studien über den Kältetod und die Kälteresistenz der Pflanzen nebst Untersuchungen über die Winterfestigkeit des Weizens. Lund, 1927.
2. Govorov, L. I. The diverse characters of winter and spring forms of cereals in connection with the problem of hardiness in winter crops. Bull. Appl. Bot. and Plant Breeding, 13: 525-559. (Russ. with Engl. abs.). 1923.
3. Martin, J. H. Comparative studies of winter hardiness in wheat. J. Agr. Research, 35: 493-535. 1927.
4. Newton, R. and Brown, W. R. Frost precipitation of proteins of plant juice. Can. J. Research, 5: 87-110. 1931.
5. Newton, R. and Brown, W. R. Catalase activity of wheat leaf juice in relation to frost resistance. Can. J. Research, 5:333-336. 1931.
6. Newton, R., Brown, W. R. and Anderson, J. A. Chemical changes in nitrogen fractions of plant juice on exposure to frost. Can. J. Research, 5:327-332. 1931.

PREPARATION OF GLUTENIN IN UREA SOLUTIONS[1]

By W. H. Cook[2] and C. L. Alsberg[3]

Abstract

A new method has been developed for preparing glutenin, using concentrated urea solution as a dispersion medium. The starch is removed from the dispersion by passing it through a Sharples supercentrifuge. The glutenin is then removed from the gliadin by precipitation: (a) by adding magnesium sulphate to about 0.17 of saturation; or (b) by adding water until the urea is diluted to about 10% concentration. Alcohol precipitation is unsatisfactory, since the glutenin loses much of its original solubility. Drying, even at room temperature, renders glutenin insoluble in 30% urea solution. Different samples of glutenin isolated by the urea method have similar amide and arginine nitrogen contents. The amount of these constituents is intermediate between the values reported for glutenin isolated from alkali, and that isolated from acid. While urea solutions denature some proteins, they affect glutenin less than dilute alkalies, as judged by the sulphydryl test, and no more than dilute acids. Neutral urea solutions permit a study of the physical properties of glutenin without previous exposure to extremes of hydrogen ion concentration. This method should be applicable to the isolation of glutenins from other seeds.

Introduction

Glutenin is described throughout the literature as being insoluble in neutral solutions. It is consequently not surprising to find that the methods described for its isolation involve a temporary dispersion in acid or alkali. Ordinarily they also include an extraction with alcohol, to remove the gliadin with which it is associated. Since the above reagents are known to denature other proteins it seems probable that glutenin, isolated by the customary methods, may have undergone changes which significantly alter its physical properties, if not its chemical composition. Since the physical properties of glutenin are regarded as of primary importance in determining the baking quality of flour, an attempt has been made in the present investigation to find a neutral dispersion medium which could be employed for the preparation and subsequent study of glutenin in its normal isoelectric state. Concentrated solutions of urea appear to fulfil the necessary conditions.

Protein Denaturation

A decrease in solubility is the first indication that a protein is altered by a given treatment. The generally accepted view as to the nature of this process is that of Hardy (20) and Chick and Martin (7) who conclude that it takes place in two stages. The first of these is termed denaturation, and although its exact nature is obscure, it is regarded as a change in the chemical structure of the protein molecule. It is not yet known whether the various forms of denaturation represent identical changes. The second stage is the flocculation

[1] *Contribution from the Food Research Institute and Department of Chemistry, Stanford University, California, and the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. The present paper is being issued as Paper No. 25 of the Associate Committee on Grain Research. It constitutes part of a thesis presented by the senior author to the Department of Chemistry, Stanford University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.*

[2] *Junior Research Biologist, National Research Laboratories of Canada.*

[3] *Director, Food Research Institute, Stanford University, California.*

of the denatured protein particles. This process is a general colloidal phenomenon, which proceeds most rapidly at the isoelectric point and in the presence of electrolytes.

The amount of protein which becomes insoluble, under conditions which favor flocculation, is generally taken as a measure of the denaturation resulting from a given treatment. Glutenin has not been considered amenable to study in this manner since it has generally been regarded as insoluble in neutral solutions. The denaturing influence of dilute acids and alkalies on this protein must therefore be inferred from investigations on other proteins.

Lewis (31, 32) found that the rate of heat denaturation of egg albumin and hemoglobin was minimal when the hydrogen and hydroxyl ion concentrations were equal. He also found (33) that high salt concentrations actually depressed the velocity of denaturation, a result in agreement with the earlier work of Chick and Martin (8). Wu and Yen (49) have presented considerable evidence which shows that dilute acids and alkalies denature many proteins.

Sjögren and Svedberg (43) have studied the stability of egg albumin solutions at different hydrogen ion concentrations with an ultracentrifuge. They found that between pH 4.0 and 9.0 the protein was stable and homogeneous in molecular weight, but beyond this range some of the molecules split into non-centrifugable substances. At pH values lower than 3.0 an increased sedimentation indicated the formation of aggregates of denatured protein.

The pH stability region of keratin has been investigated by Speakman and Hirst (44). They measured the resistance of wool to extension in different media, and conclude that it is completely stable from pH 4.0 to at least pH 7.0. The stability of both soluble and insoluble proteins appears therefore to be restricted to a limited range of hydrogen ion concentrations.

Prolonged exposure of proteins to acids and alkalies may cause chemical changes of a more complicated nature than those involved in denaturation. Anson and Mirsky (2), for instance, mention that alkali, after denaturing a protein, causes further changes which make the denatured material more soluble. Wu and Yen (49) report that dilute acids and alkalies, even at room temperature, cause the liberation of non-protein substances from proteins, including some of cereal origin.

In connection with the effects of acids and alkalies on glutenin, Blish (4) has shown that Halton's (19) success in fractionating this protein by precipitation with acid was doubtless the result of a partial racemization of the protein during the course of its preparation. Not only did a loss n optical rotatory power result, but Blish observed a change in the isoelectric point.

In a recent paper Blish and Sandstedt (5) have called in question the alkali dispersion method of preparing glutenin, by showing that different concentrations of alkali yielded fractions of different chemical composition. In these preparations the diamino nitrogen of glutenin increased with the concentration of alkali used in its preparation, while the arginine nitrogen in preparations from acid was lower than those prepared from alkali. These workers believe that the so-called 'glutenin' prepared by this method is a product resulting from the action of alkali on a more complex protein substance.

Similar evidence has been observed by Knaggs and Schryver (27) working with collagen. They found that the Hausmann numbers of gelatin depended on the previous treatment of the collagen, an acid treatment favoring a low diamino nitrogen and an alkali treatment the reverse. Later (28), they found that gelatin, which had been treated with cold dilute acids or alkalies, showed an increase in the monoamino nitrogen of the filtrate from the diamino nitrogen. Knaggs (26) reports that treatment of collagen with alkali or acid, at the hydrogen ion concentration which gives maximum swelling, will result respectively in a maximum or minimum amount of diamino nitrogen on hydrolysis.

The foregoing citations by no means exhaust the literature on the effects of dilute acids and alkalies on proteins. However, they suffice to show that both the physical properties and chemical composition of proteins, including glutenin, may be altered significantly when these reagents are employed for isolating the material. It also seems probable that the colloidal properties of glutenin are altered by the alcohol treatments used to remove the gliadin, since it is well known that alcohol denatures proteins. These considerations lead to the development of a new method of preparing glutenin, using a neutral dispersion medium.

Urea Solution as a Neutral Dispersing Agent

Two types of substances were considered as possible neutral dispersing agents: (1) solutions of the lyotropic salts, such as potassium iodide and potassium thiocyanate; and (2) solutions of certain organic nitrogenous compounds, such as urea.

Regarding the former, Gortner, Hoffman and Sinclair (18) reported that about two-thirds of the protein in flour is dispersed by a normal solution of potassium iodide. Stimulated by this work, the present authors, prior to this investigation, attempted to disperse washed gluten in 30% potassium iodide. Only part of the gluten was dispersed, hence the use of lyotropic salt solutions was abandoned.

The dispersing power of urea solutions has been observed by several workers. Spiro (45) has shown that this substance raises the coagulation temperature of proteins. Ramsden (40) however, was probably the first to demonstrate that strong urea solutions had a solvent action on proteins. Fischer (14) has also shown that dilute solutions stimulate markedly the swelling of fibrin. Recently, the solvent action of urea solutions on proteins has been demonstrated by Dill and Alsberg (13) and Burk and Greenberg (6), while Foulger (15) has shown that it has a similar effect on starch. Since Alsberg and Perry (1) had observed that concentrated urea solutions would disperse gluten, it was chosen for further consideration.

The denaturing effect of concentrated urea solutions on certain proteins has also been demonstrated. Anson and Mirsky (2) and Burk and Greenberg (6) have shown that hemoglobin and egg albumin are denatured by urea. Hopkins (24) has shown that the serum proteins, as well as egg albumin, are altered by urea solutions. The nature of this alteration appears to depend somewhat on the character of the protein. Burk and Greenberg (6) found that hemo-

globin dissolved in urea solution had a molecular weight one-half of that obtained in glycerol or water solutions in which it is not denatured. They also report that the molecular weight of casein and edestin in urea solution was only one-third and one-fourth, respectively, of the values reported by Svedberg (46), who used a centrifugal method, but it is not known whether or not these proteins were denatured by the urea. However, the former investigators and Huang and Wu (25) found that egg albumin was not altered in molecular weight when denatured by urea. Hopkins (24) and Ramsden (41) have presented evidence which suggests that urea denaturation involves an alteration in the chemical constitution of the protein. These investigators found that, in addition to the loss of solubility characteristic of denaturation, the denatured protein gives a marked sulphydryl test with nitroprusside. The temperature coefficient of urea denaturation was found to be negative, but at all temperatures the denaturation rate was sufficient to complete the change within a few hours.

Doubtless the changes involved in urea denaturation would be less obscure if the nature of the dispersing action of these solutions were better understood. Starch and many proteins, irrespective of class, are dispersed by urea. Spiro (45) explained this as due to the formation of alkali proteinates, the urea acting as an alkali. Fischer (14), on the other hand, presents evidence to show that the hydrating effect of urea is not a simple alkali effect, as acids do not counteract it in any concentration. Further evidence against the alkali theory has been presented by Dill and Alsberg (13). A possible explanation of the dispersing power of urea solutions is that they are highly polar, as shown by their high dielectric constant (16, 29). Since proteins themselves possess high polarities (17, 34), a mutual solubility might be expected. If this hypothesis is correct, the splitting of hemoglobin into fractions of lower molecular weight may be regarded as a physical phenomenon. This agrees with the opinion expressed by Burk and Greenberg (6), who consider that the mildness of urea precludes the possibility of an hydrolysis of the same type caused by acids, alkalies, or enzymes. The fact that the dielectric constant and the rate of denaturation both decrease as the temperature rises may be significant in this connection.

Most of the evidence that has been presented on the denaturation of proteins by urea solutions has appeared since this investigation was undertaken. However, urea solutions still appear to be better than acid or alkali solutions for the preparation of glutenin, because they permit a study of the protein in its isoelectric state, the condition in which it possesses the properties important in bread dough. Further, there appear to be fewer progressive secondary changes in protein in urea solution than in acid or alkali solution. In this respect, Burk and Greenberg (6) have shown that the molecular weight of casein remained constant for several days at 0° C., and Dill and Alsberg (13) found that the optical rotation of gliadin in 30% urea solution remained constant for 7 days at 37° C.

None of the other neutral solvents that might be used for dispersing proteins appear to be any better than urea, and all that have been tested caused

denaturation. Anson and Mirsky (2) have demonstrated that the lyotropic salts, potassium iodide and potassium thiocyanate, caused denaturation, while Hopkins (24) has shown that other nitrogen compounds similar to urea also affect the proteins. If proteins, such as glutenin, which are not dispersed by mild neutral solvents, obtain their insolubility from their state of aggregation, and if the action of reagents which disperse them is to break up these aggregates, then, judging from the action of urea on hemoglobin, all such proteins when dispersed will be denatured. Further, the recent work of Mirsky and Anson (35) suggests that the denaturation is not an entirely irreversible process, as it was once considered. Observations on the denaturing effect of urea on glutenin, as well as its solvent properties, were made in the present investigation.

Experimental

Methods of Analysis

Hydrogen ion concentration. This determination was made with a quinhydrone electrode at 25° C. A hydrogen electrode of the bubbling type could not be used with the urea solutions as the hydrogen appears to remove carbon dioxide, causing a drift in the alkaline direction.

Moisture. This determination was made in two ways: by heating at 98° C. for 48 hr. *in vacuo*, and by drying to constant weight over phosphorus pentoxide *in vacuo* at room temperature. Both methods showed close agreement.

Nitrogen. This determination was made by the Kjeldahl method. The values reported are corrected to a 'dry' basis, but, as no ash determinations were made, they are not corrected for mineral impurities.

Nitrogen distribution. This determination was restricted to the estimation of groups which could be used to determine whether a given preparation was gliadin, glutenin, or a mixture of these two proteins. Gliadin and glutenin, from a chemical standpoint, differ chiefly in their content of amide, arginine, and proline nitrogen. The first two of these can be determined with fair accuracy by modifications of the Van Slyke (47, 48) method. The proline nitrogen, however, cannot be estimated in this way with any degree of accuracy. Attempts to determine this form of nitrogen more directly, using a modification of Dakin's (11, 12) butyl alcohol extraction method, were also abandoned as inaccurate. Consequently, the basic, total filtrate, and filtrate amino nitrogen were determined in the usual manner by precipitation with phosphotungstic acid, although this method is subject to considerable error. A brief description of the procedure will now be given.

The proteins were hydrolyzed for 24 hr. with 6 cc. of 20% hydrochloric acid per gram of protein. The hydrolysate was then evaporated to remove most of the acid, diluted, and the acid-insoluble humin filtered off. The hydrolysate was then made up to such a volume that it would contain approximately 1 mg. of nitrogen per cc. A small amount of toluene was added as a preservative. As subsequent analyses were, in most cases, carried out on 100-cc. portions of this hydrolysate, adequate replication of all the analyses could be made from one hydrolysis. The amide nitrogen was determined by the aëration method recommended by Plimmer and Rosedale (38), except that sodium

carbonate was used instead of lime. Arginine determinations were made directly on this residue by the method of Plimmer and Rosedale (39). The precipitations with phosphotungstic acid were carried out on other residues from the amide determination in essentially the same manner as that recommended by these authors (38). The precipitate of basic nitrogen was transferred directly to a Kjeldahl flask, since no attempt was made to estimate the individual bases. Arginine determinations were carried out on an aliquot portion of the filtrate and the basic and filtrate fractions corrected for the unprecipitated arginine. No correction was applied for the solubility of the other bases.

Source of Proteins

The flour, from which the following protein preparations were made, was milled in the laboratory from a high-grade sample of Marquis, a variety of hard red spring wheat. This flour was free from bleaching agents and chemical improvers.

Preliminary Experiments

The hydrogen ion concentration of a number of urea solutions was determined at the outset, since they tend to decompose and become alkaline even at room temperature. A quinhydrone electrode gave 6.6 and 7.3 as the extreme pH values for the samples tested. In making the protein preparations, urea solutions more alkaline than pH 6.8 were adjusted to this value by the addition of dilute acetic acid. It was found that the hydrogen ion concentration of a 30% urea solution did not change significantly on standing two days. This gave ample time for centrifuging dispersions and making the other manipulations necessary at room temperature. Either Baker's or Merck's C.P. urea was used in the various experiments.

It was found that gluten could be dispersed in urea solutions of over 20% concentration. However, the lower concentrations dispersed the gluten so slowly that a 30% solution was adopted. The moisture content of a number of well-washed glutens from this flour was found to be about 60%. This was assumed to be the moisture content of subsequent glutens, and enough solid urea was added to each dispersion to bring the water contained in the gluten up to a concentration of 30% urea.

A concentrated dispersion of gluten in urea solution has a yellow, milky appearance, which remains opalescent on diluting the dispersion with more 30% urea. In the preparation of glutenin by the alkali method, Osborne (36) strongly emphasizes the necessity of filtering the dispersion clear to remove the impurities. This was attempted with the urea dispersion, but was found impossible. Either the dispersions would not filter, or, if they did, they yielded only a small amount of clear liquid which contained very little protein.

Gluten which had been extracted with alcohol to remove the gliadin was found to be entirely insoluble in 30% urea solution. To avoid this prolonged exposure to alcohol the gluten was dispersed in urea solution and alcohol added to precipitate the glutenin. Precipitation took place at an alcohol concentration of about 70% (by volume) of ethyl, and at about 60% of methyl alcohol, but only part of the resulting precipitates could be redispersed in urea. Since

Hardy and Gardner (21) report that the serum proteins can be purified with alcohol without being coagulated at temperatures of about 0° C., experiments were performed in which the precipitate was kept below 5° C. until the alcohol was removed. The precipitate obtained in this way was more soluble in 30% urea, but the amount of insoluble material was still too large to make this a feasible method for isolating glutenin.

The next method employed for precipitating the glutenin from a urea dispersion of gluten was that of 'salting-out', the method commonly used for separating serum albumin and globulin. Csonka and Jones (10) have applied this method of precipitation to alkali dispersions of gluten. They report that the gliadin and most of the glutenin is precipitated from a 0.2% solution of sodium hydroxide at a salt concentration of 0.018 of saturation with ammonium sulphate. The rest of the glutenin, which is comparatively small in amount, is precipitated at a salt concentration of 0.18 of saturation. It was thought that a salt of lower precipitating power than ammonium sulphate might facilitate the separation of the gliadin and glutenin. Magnesium sulphate was therefore selected for trial.

To determine the concentration of magnesium sulphate required to precipitate the protein from 30% urea solution, an approximately 5% dispersion of gluten was divided into 100-cc. portions and adjusted to various salt concentrations by the addition of 30% urea saturated with magnesium sulphate. The salt concentration at which the first precipitation took place is given in Table I. This precipitation coagulated all the yellow, opaque material, and left an almost clear supernatant liquid which gave a copious precipitate on the addition of tannic acid. This experiment was carried out at room temperature and the pH of the solution was 6.9 before adding the salt solution and 6.8 after its addition.

The values reported in Table I cannot be regarded as applying under all conditions. Doubtless, the salt concentration required to cause precipitation varies with the urea concentration, the hydrogen ion concentration, and the temperature at which the precipitation is carried out. The effect of these factors was not studied. Later, in making preparations by this method, it was found difficult to obtain any coagulation below a salt concentration of

TABLE I

CONCENTRATION OF MAGNESIUM SULPHATE REQUIRED TO PRECIPITATE GLUTENIN FROM 30% UREA DISPERSIONS OF GLUTEN*

Sample No.	Salt concentration expressed in terms of saturation	Time required for precipitation	Sample No.	Salt concentration expressed in terms of saturation	Time required for precipitation
1	0.13	Overnight (partial)	5	0.16	2 hr.
2	0.14	Overnight	6	0.17	15 min.
3	0.15	2 hr	7	0.18	15 min.
4	0.15	2 hr.	8	0.19	Immediately

**These samples were made up from ordinary washed gluten containing some starch.*

0.15 of saturation. The presence of starch may also influence the precipitation limit, as gluten dispersions free from starch were, in later work, found to require a salt concentration of 0.18 to bring about a satisfactory precipitation in one hour.

The protein precipitated by magnesium sulphate, in the concentrations reported in Table I, was almost entirely insoluble in 60% alcohol. This was taken as initial proof of its being glutenin. Furthermore, the supernatant liquid gave no precipitate when adjusted to an alcohol concentration of 70% by volume, this indicating that the protein which remained dispersed was largely gliadin.

The efficacy of this method of separating the two proteins depends on the salt concentration at which gliadin starts to precipitate. To test this point, a sample of gliadin was dissolved in 30% urea in 1, 3, 7 and 10% concentrations. All of these dispersions were perfectly clear. A solution of 30% urea saturated with magnesium sulphate was then added to these dispersions, and the salt concentrations which caused (a) permanent turbidity, and (b) definite slight precipitation, were noted. The results obtained are reported in Table II. It is evident that the point of permanent turbidity can be more accurately determined and is the more important, because gliadin in that condition would be swept down by the flocculating glutenin.

TABLE II

CONCENTRATIONS OF MAGNESIUM SULPHATE REQUIRED TO PRECIPITATE GLIADIN FROM 30% UREA SOLUTIONS

Concentration of prepared gliadin, %	Salt concentration expressed in terms of saturation	
	Permanent turbidity	Definite slight precipitation
1	0.15	0.18
3	0.14	0.18
7	0.14	0.16
10	0.13	0.14

The results given in Table II show that the salt concentration required to cause permanent turbidity depends somewhat on the concentration of the gliadin. However, all concentrations become turbid at a salt concentration below that required to precipitate glutenin. This result suggests that the lowest salt concentration which will cause precipitation of the glutenin will be most effective in removing the gliadin. In subsequent work, each dispersion of gluten was titrated to the salt concentration that would just give precipitation of the glutenin in from one to two hours.

The glutenin precipitated by salting-out was readily dispersed by 30% urea solution after washing out the salt with distilled water. A small amount of denatured material would result from each salt precipitation but it was much less than that obtained by precipitation with alcohol. Glutenin which had been purified by three precipitations with salt was allowed to stand in 60% alcohol for extended periods, with occasional shaking. The first extracts so

obtained gave a faint test for gliadin. As subsequent extractions with 60% alcohol gave negative tests for soluble nitrogen it was concluded that salting-out was an effective method of separating the two proteins.

It was found that the urea could not be completely removed from the last protein precipitate by extraction with 60% alcohol at room temperature. If the precipitate was exhaustively washed with water, however, the nitrogen content of the protein indicated the absence of urea. The possibility of urea remaining sorbed on the protein was tested further by extracting the washed protein with water at 60° C. for several hours. Tests for urea made on the aqueous extracts thus obtained were negative, indicating that the adsorbed urea which survived the washing would not affect appreciably the chemical composition of the protein.

Even when the gluten was extremely well washed, the dispersions showed the presence of considerable amounts of starch. This would settle out partly on standing several days, but even then the dispersion gave a strong test for starch. Ordinary centrifuging was of little value in reducing this starch test, so the starch was regarded as being dispersed, in accordance with the work of Foulger (15), and its removal at this stage was abandoned in favor of its removal later by precipitation. In isolating the glutenin by salting-out, the strong starch test of the original gluten dispersion was reduced to a faint test in the dispersion obtained from the first precipitate, while the dispersion from the second precipitate usually gave a negative starch test. This suggested that the starch was less soluble than the glutenin, and remained behind in the small amount of insoluble material. Nevertheless the nitrogen content of these preparations was usually lower than the 17.49% reported by Osborne (37) for glutenin. However, as the preparations appeared to be free from starch, no further improvements in the method were attempted at this stage.

Several efforts were made to obtain dry glutenin which would redisperse in 30% urea. All the samples that were dehydrated with alcohol and ether were denatured, as has been noted previously. Drying at low temperature without the use of alcohol resulted in a similar insolubility. Two small preparations were then dried carefully at room temperature, using an air blast and grinding the material through a food chopper several times to facilitate drying. When tested in the air-dry condition, they were found to be entirely insoluble in 30% urea solution. As these preparations were originally quite soluble, the only apparent denaturing influence was that of drying the material. This suggests that the change involved in the denaturation of glutenin may be some form of dehydration.

Description of Preparations

Having given the method of preparation some study, the isolation of a sample of glutenin large enough for subsequent analysis was undertaken.

Preparation 1. About 300 gm. of well-washed wet gluten was torn up and placed in 1700 cc. of 30% urea solution, and sufficient solid urea added to bring the water in the gluten up to a urea concentration of approximately 30%. This was allowed to stand for 24 hr. at room temperature with occasional

stirring. The small amount of undispersed material was removed by centrifuging and the dispersion adjusted to a salt concentration of 0.14 of saturation by adding 30% urea saturated with magnesium sulphate. At this salt concentration, no immediate coagulation was noticeable, but it precipitated on standing overnight, leaving a slightly turbid supernatant liquid which was decanted. The residue was then washed repeatedly with water, replaced in 1500 cc. of urea solution (again adding sufficient solid urea to adjust the water in the precipitate to approximately 30% urea), allowed to stand 24 hr. to disperse, and then centrifuged to remove the insoluble material. As the starch test in this dispersion was almost as marked as in the original dispersion, sufficient salt solution was added to make the dispersion 0.16 saturated, to see if this would render the carbohydrate impurities less soluble. Precipitation occurred immediately, leaving a clear supernatant liquid. The precipitate was washed with water several times, redispersed in 30% urea, and finally centrifuged to remove the insoluble material. The starch test was much weaker this time, and after another precipitation at 0.16 of saturation, this test was negative.

The supernatant liquid from this precipitation was adjusted to a salt concentration of 0.20 of saturation. A small quantity of protein was precipitated, which was washed and dehydrated. This sample gave a negative starch test, a positive Molisch test, and contained 16.7% nitrogen.

The precipitate obtained at 0.16 of saturation was redissolved in 30% urea and precipitated by dilution, using five volumes of distilled water. This method of precipitation was used to facilitate the removal of the magnesium sulphate and urea. The precipitate came down in a white flocculent form, which was easily broken up by shaking. It was washed with water until free from the sulphate ion and urea. The sample thus obtained was extracted for two days with 60% alcohol, the water in the precipitate having been adjusted to this concentration with 95% alcohol. This extract gave a faint test for gliadin, but a second 60% alcohol extract was protein-free. The sample was then dehydrated with absolute alcohol and anhydrous ether, and finally dried over phosphorus pentoxide *in vacuo*. The sample obtained was a white, finely divided powder. It weighed nine grams, which corresponds to about 10% of the weight of dry gluten used. This preparation gave a negative starch test, a positive Molisch test, and contained 16.4% nitrogen. A further analysis of this sample is given in Table IV.

Judging from the appearance of the protein, the single precipitation by dilution in the last preparation effected more purification than a single precipitation with salt. If this method were used throughout, however, the removal of gliadin would depend on its solubility in dilute solutions of urea. Dill and Alsberg (13) tested the solubility of 1% of gliadin in urea solution and obtained a turbidity on dilution to 2.03 molar (12%). Observations made during the course of this investigation suggested that gliadin was soluble in urea solutions more dilute than this. A test, conducted to determine the solubility of gliadin in 10% urea, showed that this urea concentration would disperse 5% of gliadin to an extremely viscous, clear sol. On dilution, this dispersion

became turbid, but the resulting precipitate represented only part of the original protein.

These results with prepared gliadin made it appear that not only was precipitation by dilution a feasible method of separating the two proteins, but that an initial extraction with 10% urea would be helpful in removing the gliadin. A test preparation made by extracting gluten six times with 10% urea solution, followed by two dispersions and dilution precipitations from 30% urea, yielded a sample which gave a negative test for starch and contained 16.4% of nitrogen. The amide and arginine nitrogen contents of this preparation were respectively 20 and 10% of the total nitrogen.

Preparations 2 and 3. The preparation of a larger sample by this method was then undertaken. Unintentionally, the protein was fractionated when the first precipitation was made, resulting in two preparations of glutenin.

These preparations were started by extracting 600 gm. of wet gluten with 10% urea solution. Six extractions were made, using about 1500 cc. of urea solution for each extraction, which was of 12 hr. duration, the material being stirred frequently. After the sixth extraction, 2500 cc. of 30% urea solution was added, and the remaining material allowed to disperse for 24 hr. at room temperature. A small amount of insoluble material was then thrown down in an ordinary centrifuge, and the dispersion diluted with two volumes of distilled water. As no precipitation took place, another volume of distilled water was added, thus diluting the urea concentration to about 7.5%. As this did not cause precipitation, the equivalent of 0.5 gm. per litre of sodium chloride was added. This gave a white flocculent precipitate which was removed by decantation, and is subsequently designated as Preparation 2.

The supernatant liquid from the above precipitation was quite opaque and appeared to contain considerable protein. A further addition of 0.5 gm. per litre of salt resulted in a precipitate similar to the first. This also was obtained by decanting off the liquid, and was kept separate from the first precipitate and designated Preparation 3.

After the removal of the second precipitate, the supernatant liquid was not entirely clear, so a third addition of salt was made. A small amount of precipitate resulted, which was purified and dehydrated, but was not allotted a preparation number as it was later found to be largely gliadin. The first indication of this was that much of it was lost in subsequent purification owing to its solubility in 10% urea. The product obtained from this third precipitation, when dehydrated, was found to contain only 15.7% nitrogen, which indicated the presence of impurities, although the starch test was negative. A small amount was hydrolyzed and yielded 27.1% of its nitrogen as ammonia, which definitely established that it was gliadin.

The subsequent purification of Preparations 2 and 3 will now be described together, since it was essentially the same for both samples. The precipitates from the fractional precipitations were dissolved in urea solution, centrifuged, and again diluted with two volumes of distilled water. As no precipitate came down, and since further fractionation was undesirable, 2 gm. per litre of salt was added. This gave immediate precipitation, leaving a

clear supernatant liquid. Both precipitates were then washed with 10% urea solution. As the first two of these washings dispersed sufficient protein to make them quite opaque, they were precipitated and purified separately. The results of such analyses as could be performed on these samples are shown in Table III, together with similar analyses of the preparation from which they were obtained.

After four washings with 10% urea solution, the material from the second precipitation was redissolved in 30% urea, centrifuged, and reprecipitated as before, except that lithium chloride instead of sodium chloride was emplo ed. The precipitates were then washed once with 10% urea, which appeared to remove but little protein, and then with water until the washings gave no test for the chloride ion or urea. The samples were then dehydrated with alcohol and ether. The 60% alcohol extractions gave a faint test for gliadin on the first washing only. Other tests conducted on the dry samples are reported in Table III, and a more comprehensive analysis given in Table IV.

TABLE III

STARCH AND MOLISCH TESTS AND NITROGEN CONTENT OF PREPARATIONS 2 AND 3 AND EXTRACTS OBTAINED FROM THEM

Nature of sample	Preparation 2			Preparation 3		
	Starch test	Molisch test	Nitrogen content, %	Starch test	Molisch test	Nitrogen content, %
Protein in 1st 10% urea extract	−	?	17.0	−	?	17.0
Protein in 2nd 10% urea extract	−	?	16.7	−	?	16.7
Purified preparation	−	+	15.8	−	?	16.3

It is evident from Table III that the nitrogen contents of the proteins extracted by 10% urea solution are higher than those of the purified preparation. This suggests that some impurity of lower solubility than the glutenin was present in the material. Tests for starch were negative, but Preparation 2 gave a positive Molisch reaction. The rest of these fractions, when tested for carbohydrate material, gave such a faint test that the presence of impurities of this nature could not be definitely established. It is probable that the carbohydrate impurity in these preparations was the gum mentioned by Hoffman and Gortner (23).

The behavior of starch in 30% urea was given some consideration at this point. It has been mentioned that a gluten dispersion in urea gives a strong test for starch, which was regarded as being dispersed, since no appreciable amount could be settled out by prolonged centrifuging in an ordinary centrifuge. To test this assumption, a sample of starch prepared from the same flour was placed in 30% urea solution. This starch swelled considerably, but even on standing ten days with occasional shaking, a water-clear supernatant liquid remained. This clear liquid gave a test for starch but it

appeared to be small in amount compared with the undispersed material. The starch, and other impurities affecting the nitrogen contents of Preparations 2 and 3, appear, therefore, to be less soluble than the protein. This result, together with the data presented in Table III, indicated that the carbohydrate impurities were less easily dispersed than the protein. It should, therefore, be possible to remove them. Ordinary centrifuging, however, was of little value; filtering the dispersion was so slow as to be impracticable, and also appeared to remove most of the glutenin.

At this stage, a Sharples supercentrifuge was made available for this work. A gluten dispersion passed through this machine at a rate of 50 cc. per minute gave a negative starch test, and on subsequent purification the glutenin so obtained contained 17.1% of nitrogen. As this procedure was apparently effective in removing the impurities, the preparation of further samples was undertaken.

Preparation 4. A dispersion of gluten was prepared by placing 400 gm. of wet gluten, plus the calculated amount of solid urea, in four litres of 30% urea solution. This was stirred for two hours at room temperature, and then allowed to stand for 18 hr. at 0° C. in a refrigerator. The dispersion was then supercentrifuged, as described above. About 4200 cc. of a starch-free protein solution was thus obtained. This was divided into two equal parts, one of which was used for making Preparation 5, to be described later.

Preparation 4 was made by salting-out the glutenin. The dispersion described above was adjusted, at intervals of about one hour, to salt concentrations of 0.14, 0.15, 0.16, 0.17, and finally to 0.18 of saturation, at which salt concentration flocculation took place. This precipitate was allowed to settle overnight at 0° C. It was then washed three times with distilled water and redispersed in 1500 cc. of 30% urea. The protein dispersed readily, but it was allowed to stand 12 hr. before supercentrifuging it again. This removed a small amount of material which appeared to be protein. Precipitation of this dispersion took place at a salt concentration of 0.16 of saturation. The precipitate was allowed to settle, washed, redispersed, supercentrifuged, and again precipitated at a salt concentration of 0.16 of saturation. It was then washed with water until free from urea and the sulphate ion. This was followed by three prolonged extractions with 60% alcohol, the first two of which gave a faint test for gliadin. The preparation was then dehydrated. About six grams of protein was obtained, which is a lower yield than that obtained in Preparation 1. This agrees with the observation that more of the precipitated material became insoluble during the purification process. This may be attributed to the higher salt concentration used for the first precipitation, or to the more effective removal of the denatured material by the supercentrifuge. The nitrogen content of this preparation was 17.0%; further analyses are given in Table IV.

Preparation 5. This preparation was obtained by precipitating the glutenin by dilution. Half of the starch-free dispersion described under Preparation 4 was used. To avoid fractionation in the first precipitation, this dispersion was poured into three volumes of 1% lithium chloride solution. Precipitation

took place immediately as a coherent, gluten-like mass, which could not be washed satisfactorily; so after two attempts, it was redispersed in 30% urea solution. After allowing 24 hr. for complete dispersion, it was supercentrifuged and precipitated by pouring it into three volumes of distilled water. This also resulted in a gluten-like precipitate, which could not be washed readily. Apparently, this coherent precipitate was partly due to the large amount of gliadin present, which had been to a large extent removed from Preparations 2 and 3 by the initial extraction with 10% urea. This precipitate was then washed twice with 10% urea before replacing it in the more concentrated solution. On standing overnight, dispersion was complete and it was again supercentrifuged and precipitated by the careful addition of water to the dispersion. This gave a fine, white precipitate, which flocculated readily. The urea concentration at which this precipitation occurred was approximately 12%. The precipitate was washed with 10% urea and subjected to one more dispersion and precipitation before washing it free from urea and dehydrating it in the usual manner. The first two extractions with 60% alcohol gave a faint test for gliadin. Somewhat more denaturation was observed in this preparation than in Preparations 2 and 3. About five grams of dry protein was obtained, which contained 17.3% nitrogen. Further analyses are reported in Table IV.

Preparation 6. A small amount of glutenin was isolated by solution in alkali for comparing the nitrogen distribution of such a preparation with those isolated by dispersion in urea solutions. After exhaustive extraction of the gliadin with 60% alcohol, the remainder of the gluten was dispersed in 0.2% sodium hydroxide. This dispersion was supercentrifuged and the protein precipitated by neutralization with 0.5 *N* hydrochloric acid. Three such precipitations were made in all, each precipitate being washed with 60% alcohol before redissolving in alkali. This preparation also gave a faint test for gliadin in the first two 60% alcohol washings from the final precipitate. This preparation contained 16.4% nitrogen.

Analytical Results

The values for the nitrogen content and nitrogen distribution of the glutenin preparations just described are given in Table IV. Preparations 4 and 5, which were supercentrifuged to remove the starch and other impurities, have distinctly higher nitrogen contents than the first three preparations. The reported values are on a moisture-free basis, but corrections were not made for their mineral content. Ash determinations on Preparations 4 and 5 showed less than 0.4% ash, an amount which would not affect the nitrogen percentages appreciably. Osborne (37) gives the nitrogen content of glutenin as 17.49%, which is significantly higher than any of these preparations. It must be concluded either that these preparations contained non-nitrogenous impurities, or that Osborne's value is too high. The latter is at least possible, since the prolonged alcoholic extraction of the older methods of preparation might have denatured some of the less soluble fractions of the gliadin.

TABLE IV

NITROGEN CONTENT AND DISTRIBUTION OF GLUTENIN PREPARATIONS

Preparation No.	Dispersion medium and method of preparation	Nitrogen content	Nitrogen distribution								
						Phosphotungstic acid precipitation					
							Values for fractions corrected for arginine nitrogen in filtrate				
			Humin N	Amide N	Arginine N	Arginine N in filtrate	Basic N	Total filtrate N	Filtrate amino N	Filtrate non-amino N	Recovery
		%	%	%	%	%	%	%	%	%	%
1	Urea dispersion salted out	16.4	0.6	16.4	11.1	2.7	21.4	62.1	54.7	7.4	99.9
2	Urea dispersion diluted	15.8	1.3	19.4	9.9	2.5	18.6	61.0	57.4	3.6	99.0
3	Urea dispersion diluted	16.3	0.7	19.9	9.6	2.7	17.9	60.8	54.8	6.0	99.3
4	Urea dispersion salted out*	17.0	0.3	19.3	9.8	5.0	17.5	61.2	60.7	0.5	98.3
5	Urea dispersion diluted*	17.3	0.4	19.5	10.0	3.8	17.8	61.6	60.3	1.3	99.3
Average of preparations 2, 3, 4 and 5				19.5	9.8		18.0	61.2			
6	NaOH neutralized	16.4	0.5	17.9	11.0	5.0	23.2	57.7	57.7	0.7	99.3

These preparations were supercentrifuged to free them from carbohydrate and other impurities.

All values given in Table IV for the nitrogen distribution are expressed as a percentage of the total nitrogen in the hydrolysate. It is evident that the last four urea preparations (Preparations 2 to 5) are essentially the same in their contents of amide and arginine nitrogen. They are also similar in their basic and total filtrate nitrogen contents, although less accuracy is claimed for these figures. The variations between these preparations in filtrate amino nitrogen may signify real differences, but, considering the errors inherent in such a determination, and the similarity of the preparations with respect to the other nitrogen fractions, the variations are probably not significant. Since the non-amino nitrogen is subject to more error than any of the other fractions, its variability in the different preparations cannot be taken as establishing the fact that there are any real differences between them.

Preparation 1 differs significantly from the other urea preparations in having a lower amide and a higher arginine nitrogen content, resembling in this respect the alkali preparation (Preparation 6). There is no evident reason for this preparation differing from the others, but it is probable that it was denatured or otherwise altered to some extent, since it was the first sample isolated by the urea method. Blish and Sandstedt (5) have found that the amide nitrogen content of glutenin decreases, and the arginine nitrogen increases with the concentration of alkali used for its dispersion. If it can be inferred that the higher concentrations of alkali alter the glutenin more than lower concentrations, then a decrease in amide nitrogen and an increase in arginine nitrogen represent a greater degree of alteration. From this reasoning, Preparations 1 and 6 appear to be altered in comparison with the other urea

preparations. While this is probably so, no reason can be given for this alteration in the case of the urea preparation. The similarity between the arginine and amide nitrogen contents of the last four urea preparations, which includes precipitation by salting-out and by dilution, leads to the conclusion that glutenin is at least a chemical individual.

In Table V some of the values reported in the literature for the amide and arginine nitrogen of glutenin are presented for comparison with the preparations just described. The values for the other fractions obtained in the determination of the nitrogen distribution are not compared, as they are subject to greater error, especially in comparing the work of different investigators. The values obtained by any one investigator have been averaged, regardless of the source of the glutenin. This is permissible, since the individual workers have interpreted their results as showing no significant variation between the glutenins from different flours. Further, Blish and Sandstedt (5) have shown that the percentage of amide and arginine nitrogen is affected by the concentration of alkali used in dispersing the glutenin. The values for the four urea preparations having essentially the same composition in regard to the constituents concerned have also been averaged. The data obtained from Preparations 1 and 6 are presented separately.

It is evident that the composition of Preparations 1 and 6 come well within the range of values obtained by other investigators for glutenin prepared by

TABLE V

AMIDE AND ARGININE NITROGEN CONTENTS OF GLUTENIN

Observer	Method of isolation	No. of preparations analyzed	Amide N %	Arginine N %
Osborne 1909 (37)	Alkali dispersion	—	18.8	—
Blish 1916 (3)	Alkali dispersion	2	16.4	9.5
Cross and Swain 1924 (9)	Alkali dispersion	4	14.9	10.0
Hoffman and Gortner 1927 (23)	Alkali dispersion	1	13.6	12.0
Larmour 1927 (30)	Alkali dispersion	1	14.8	10.9
Csonka and Jones (α-glutelin)* 1927 (10)	Alkali dispersion	1	17.8	11.0
Blish and Sandstedt 1929 (5)	Acid dispersion	9 or 10	about 22	about 9
Authors	Alkali dispersion (Prep. 6)	1	17.9	11.0
Authors	Urea dispersion (Prep. 1)	1	16.4	11.1
Authors	Urea dispersion (Preps. 2 to 5)	4	19.5	9.8

**The values for the β-glutelin are not given as it apparently occurs in small quantity.*

the alkali method. As Preparation 6 was prepared from alkali, and Preparation 1, though isolated by the urea method, was apparently altered in some way, these preparations will not be discussed further. The amide nitrogen content of the other glutenin samples prepared by the urea method is significantly higher than all the preparations made by the alkali method. Since no urea could be detected in an aqueous extract of the protein made at 60° C., it appears that the high amide nitrogen of the urea preparations cannot be attributed to urea remaining sorbed on the protein. The glutenin preparations of Blish and Sandstedt (5), made by dispersion in acid, are the only ones having a higher amide nitrogen content than the urea preparations. Considering the arginine nitrogen of glutenin, it is evident that the urea preparations give values which are intermediate between those of the acid dispersion and those of the alkali dispersion preparations, except in the case of the alkali preparations reported by Blish (3).

It appears from these results that the hydrogen ion concentration of the dispersion medium used for preparing the glutenin has some effect on its subsequent amide and arginine nitrogen values. This agrees with the work of Knaggs and Schryver (27), and Knaggs (26) who found that acid- and alkali-treated collagens differed in their content of diamino nitrogen. This is of special interest, since collagen resembles glutenin in being insoluble in the neutral solvents commonly used for dispersing proteins. The results of chemical analyses performed on such proteins as glutenin should therefore be more reliable when a neutral dispersion medium is employed for their purification.

Possible Denaturation by Urea Solutions

Since concentrated urea solutions are known to denature certain proteins, attempts were made to determine their effect on glutenin. This is a difficult point to test experimentally by the usual solubility methods since glutenin is originally insoluble in the ordinary neutral solvents. In making the preparations just described, a small amount of protein remained insoluble after each precipitation, but this takes place in the preparation of most proteins. Physical tests for determining the denaturing effect of urea solutions on gluten were conducted by dispersing washed gluten in 30% urea solution, supercentrifuging to remove the starch, and then determining the viscosity of the dispersion after storage for various periods at 5° C. The hydrogen ion concentration was adjusted to pH 6.95 with phosphate buffer mixtures. No change in viscosity was observed for periods up to 100 hr., the longest storage time employed. There was no evidence of precipitation even when the dispersions were stored for much longer periods. It should be noted, however, that the gluten was completely dispersed before any viscosity measurements were made. According to Hopkins (24) urea denaturation takes place rapidly, and as it has a negative temperature coefficient, it is possible that the gluten was altered before the initial viscosity was observed. Further studies on viscosity changes in relation to the denaturation of the gluten proteins will be presented in a later paper.

Harris (22) and more recently, Hopkins (24) have shown that such proteins as egg albumin, when denatured either by heat or by concentrated urea solutions, give a positive test for a thiol group when treated with sodium nitroprusside. Ramsden's (42) results indicate that it is necessary to remove the urea from the protein before making the nitroprusside test for denaturation.

The first tests were made on flour and wet gluten in order to establish the validity of this test as a criterion of denaturation in the gluten proteins. The results obtained with flour were inconclusive, due to their large starch content, but if any color developed it was extremely faint. Wet gluten gave a doubtful test at first but a deeper color gradually developed on standing, a result which may be attributed to denaturation caused by the weak alkaline solution in which the test is made. This test was then applied to the preparations described in Table IV which had, of course, been denatured by drying. The urea preparations all gave a faint test, but the alkali preparation gave a more intense color than any of these. Some samples of gliadin prepared in the usual manner, which does not involve exposure to alkali or urea, gave tests of similar intensity. Further tests were made on gluten dispersed in (a) dilute sodium hydroxide, (b) dilute acetic acid, and (c) 30% urea for 48 hr. and then dialyzed. The protein from the alkali dispersion gave a much deeper color than the protein dispersed in acetic acid or urea, the two latter giving about the same shade.

Although these tests must be regarded as of a preliminary nature, they furnish evidence to show that the thiol test is indicative of denaturation in the gluten proteins. All of the samples of prepared gliadin and glutenin, subjected to test, gave a faint positive reaction, indicating that the purification process had caused some denaturation. Judging from these results, glutenin prepared by the urea method is altered less than samples prepared by solution in alkali.

Discussion and Conclusions

At the present time, it cannot be stated definitely whether salting-out or dilution is the better method of precipitating glutenin from urea dispersions. A small amount of denatured material occurs with both methods. It is probable, however, that precipitation by dilution yields a product with a lower content of mineral impurities. If this method of precipitation is employed, it appears to be advantageous to extract thoroughly with 10% urea solution first, in order to remove most of the gliadin. This renders the subsequent precipitates less coherent and consequently facilitates the washing of the material.

Whatever the method of precipitation used, it is necessary to remove the carbohydrate impurities at an early stage. This can be accomplished by passing the 30% urea dispersions slowly through a Sharples supercentrifuge. Usually three precipitations suffice to remove the gliadin from the glutenin, provided each precipitation is made under conditions which will just cause the glutenin to coagulate. Any excess of the precipitating agent will cause more of the gliadin to be thrown down with the glutenin; the latter protein in consequence will have to be precipitated more often to insure purity. When the removal of the gliadin is completed, the precipitate is washed by shaking vigor-

ously with water at 0° C. until the washings contain no measurable amount of nitrogen, and then washed several times with water at room temperature, the urea appears to be completely removed from the protein.

Glutenin loses its original solubility in 30% urea solution if it is exposed to alcohol or dried; such treatments must therefore be avoided in making preparations for physical study. Hitherto, dilute alkalies and acids have been used for the preparation of glutenin. Judging from the sulphydryl test, urea solutions cause less denaturation than dilute alkalies, and are not inferior to acid in this respect. In contrast to the alkali preparations, different samples of glutenin isolated by the urea method have practically similar amide and arginine nitrogen contents. The amount of these constituents is intermediate between those reported for preparations made by acid and alkali. Urea solutions, being neutral, also permit a study of the physical properties of glutenin in the region of its normal isoelectric condition without previous exposure to extremes of hydrogen ion concentration.

Proteins of the glutelin class occur in many of the cereal grains, such as wheat, oats, barley, corn, etc. Of these grains, only wheat yields a gluten from which most of the starch can be removed by kneading in a stream of water. In preparing the glutelins from the other cereals, it is, therefore, necessary to extract the protein directly from the flour or ground material. Since solutions of urea apparently disperse the protein more readily than the starch, it would seem that the urea method just described could be adapted to the preparation of glutelins from other cereals. The application of this method to the preparation of other glutelins would doubtless lead to a better understanding of these proteins; at least, it would avoid the alterations caused by prolonged contact with alkali and alcohol.

Acknowledgment

The authors wish to thank Dr. R. Newton, Professor of Field Crops, University of Alberta, for helpful suggestions made during the course of this investigation and for his criticism of the manuscript.

References

1. ALSBERG, C. L. and PERRY, E. E. "The preparation of glutenin". Abstract circulated for Los Angeles meeting of the American Chemical Society, Aug. 1925.
2. ANSON, M. L. and MIRSKY, A. E. J. Gen. Physiol. 13: 121-132. 1929.
3. BLISH, M. J. J. Ind. Eng. Chem. 8: 138-144. 1916.
4. BLISH, M. J. Cereal Chem. 2: 127-131. 1925.
5. BLISH, M. J. and SANDSTEDT, R. M. J. Biol. Chem. 85: 195-206. 1929.
6. BURK, N. F. and GREENBERG, D. M. J. Biol. Chem. 87: 197-238. 1930.
7. CHICK, H. and MARTIN, C. J. J. Physiol. 43: 1-27. 1911.
8. CHICK, H. and MARTIN, C. J. J. Physiol. 45: 261-295. 1913.
9. CROSS, R. J. and SWAIN, R. E. Ind. Eng. Chem. 16: 49-52. 1924.
10. CSONKA, F. A. and JONES, D. B. J. Biol. Chem. 73: 321-329. 1927.
11. DAKIN, H. D. Biochem. J. 12: 290-317. 1918.
12. DAKIN, H. D. J. Biol. Chem. 44: 499-529. 1920.
13. DILL, D. B. and ALSBERG, C. L. J. Biol. Chem. 65: 279-304. 1925.
14. FISCHER, M. H. Oedema and Nephritis, Wiley, N.Y. 1915.

15. Foulger, J. H. Med. Bull. Univ. Cincinnati, 3: 46-47. 1924.
16. Fürth, R. Ann. Physik, 70: 63-80. 1923.
17. Garreau, Y. and Marinesco, M. N. Compt. rend. 189: 331-333. 1929.
18. Gortner, R. A., Hoffman, W. F. and Sinclair, W. B. Colloid Symposium Monograph, 5: 179-198. 1928.
19. Halton, P. J. Agr. Sci. 14: 587-599. 1924.
20. Hardy, W. B. J. Physiol. 24: 158-210. 1899.
21. Hardy, W. B. and Gardner, S. J. Physiol. 40: lxviii. 1910.
22. Harris, L. J. Proc. Roy. Soc. Lond. 94B: 426-441. 1923.
23. Hoffman, W. F. and Gortner, R. A. Cereal Chem. 4: 221-229. 1927.
24. Hopkins, F. G. Nature, 126: 328-330, 383-384. 1930.
25. Huang, T. C. and Wu, H. Chinese J. Physiol. 4: 221-230. 1930. Quoted by Chem. Abs. 24, 4061. 1930.
26. Knaggs, J. Biochem. J. 23: 1308-1327. 1929.
27. Knaggs, J. and Schryver, S. B. Biochem. J. 18: 1095-1101. 1924.
28. Knaggs, J. and Schryver, S. B. Biochem. J. 18: 1102-1106. 1924.
29. Kockel, L. Ann. Physik, 77: 417-448. 1925.
30. Larmour, R. K. J. Agr. Research, 35: 1091-1120. 1927.
31 Lewis, P. S. Biochem. J. 20: 965-977. 1926.
32. Lewis, P. S. Biochem. J. 20: 978-983. 1926.
33. Lewis, P. S. Biochem. J. 20: 984-992. 1926.
34. Marinesco, M. Compt. rend. 188: 1163-1165. 1929.
35. Mirsky, A. E. and Anson, M. L. J. Gen. Physiol. 13: 133-143. 1929.
36. Osborne, T. B. Carnegie Inst. Wash. Publ. 84. 1907.
37. Osborne, T. B. The vegetable proteins. Longmans, Green. Lond. 1909.
38. Plimmer, R. H. A. and Rosedale, J. L. Biochem. J. 19: 1004-1014. 1925.
39. Plimmer, R. H. A. and Rosedale, J. L. Biochem. J. 19: 1020-1021. 1925.
40. Ramsden, W. J. Physiol. 28: xxiii-xxvi. 1902.
41. Ramsden, W. Chemistry and Industry, 49: 851-852. 1930.
42. Ramsden, W. Nature, 127: 403-404. 1931.
43. Sjögren, B. and Svedberg, T. J. Am. Chem. Soc. 52: 5187-5192. 1930.
44. Speakman, J. B. and Hirst, M. C. Nature, 127: 665-666. 1931.
45. Spiro, K. Z. physiol. Chem. 30: 182-199. 1900.
46. Svedberg, T. Kolloid-Z. 51: 10-24. 1930.
47. Van Slyke, D. D. J. Biol. Chem. 10: 15-55. 1911.
48. Van Slyke, D. D. J. Biol. Chem. 22: 281-285. 1915.
49. Wu, H. and Yen, D. J. Biochem. (Japan), 4: 345-384. 1924.

STUDIES IN THE VARIABILITY OF TUBERCLE BACILLI. IV. ANTIGENIC PROPERTIES OF S AND R CULTURES[1]

CHRISTINE E. RICE[2]

Abstract

Accompanying dissociation of certain cultures of tubercle bacilli, a correlated loss in the specific antigenic activity in the complement-fixation reaction has been noted. All extracts made from attenuated cultures in which the R colony has been shown to be predominant have been found to be inferior in antigenic activity to similar preparations made from cultures composed of S types. Antisera prepared against R organisms appear to lack antibodies against certain substances present in the S organisms, although they have been found to contain a higher proportion of antibodies reacting with related acid-fast organisms than do the corresponding S antisera. It has been necessary to employ a strictly quantitative technique to demonstrate these differences.

The work of Petroff and his associates (15, 16, 17) on the dissociation of tubercle bacilli, has been confirmed indirectly by several workers, and directly by Begbie (5) and by Reed and Rice (19, 20) in the first two papers of this series. In the latter paper, it was shown that dissociation of these organisms is accompanied by correlated changes in colony structure, in certain physical properties and in pathogenicity. Dissociation such as has been demonstrated in many bacterial species, appears to involve in addition to these changes, certain fundamental alterations in antigenic capacity, the variant type being lacking in the specific antigenic qualities which characterize the normal, freshly isolated organisms. In the colon-typhoid group of bacteria, the essential feature of the S to R change seems to be an alteration in the heat-stable somatic complex with the development of new and typical somatic antigens (White (27) and Arkwright (1, 2)). In the pneumococci and streptococci, the antigenic change would appear to be less complete, the variant although lacking the type-specific antigens, still retains the group antigen also found in the normal type (Griffith (11), Todd and Lancefield (24)). The antigenic behavior of other dissociated cultures has received less attention, but would appear to follow either of these two types of change. The present paper is concerned with a study of the antigenic behavior of certain typical S and R cultures of the tubercle bacillus.

A number of workers who have studied the complement-fixation reaction in tuberculosis have noted variation in the antigenic activity of preparations made from different strains of tubercle bacilli, Scheff (21) and Wadsworth, Maltaner and Maltaner (26) having found extracts of certain avirulent strains of these organisms to be less suitable as antigens than those made from virulent ones. Cooke (8) made the interesting observation that strains of tubercle bacilli

[1] *Manuscript received June 23, 1931.*
Contribution from the Department of Bacteriology, Queen's University, Kingston. This work was carried on with the co-operation and financial assistance of the Associate Committee on Tuberculosis of the National Research Council of Canada.
The data presented in this paper formed part of a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy at the University of Toronto.

[2] *Fellow in Bacteriology, Queen's University.*

which were readily emulsified in saline, made the better antigens, an observation possibly related to the fact that S types are somewhat more readily emulsified than R. Shibata (22), however, could find no appreciable difference in the complement-fixing activity of alcoholic extracts prepared from the virulent Vallée and the avirulent B.C.G. strain in the presence of tuberculous serum.

Ordinary serological procedures have not been as successful to date in the differentiation of types of tubercle and other acid-fast species as have cultural methods and animal experimentation. The very close antigenic relationship within this group was demonstrated by Koch in 1901 by means of the agglutination reaction and two years later by Bordet and Gengou (6) by complement-fixation. Since that time cross reactions have been reported repeatedly (Babès and Busilia (4), Harris and Lanford (12), Cooke (8), Coulthard (10), Scheff (21), Aronson and Whitney (3), and others). Smith (23) was unable to differentiate between human and bovine tubercle bacilli by means of agglutinin absorption tests, while Wadsworth, Maltaner and Maltaner (26), Kirchner (13) and Aronson and Whitney (3) have failed to do so by means of the complement-fixation reaction. It seems evident from these results that if differences in antigenic activity between the virulent and avirulent types or between the S and R forms actually exist, more precise quantitative procedures of measurement must be adopted in order to detect them.

Of the various serological methods, complement-fixation has been found to give the most reliable results both in classification of the acid-fast species and laboratory diagnosis of tuberculous infection. The quantitative technique proposed by Mrs. Maltaner (14), in which the actual amount of complement reacting with the sensitized antigen is measured, appeared to be the most exact method so far reported and was therefore considered most suitable for our purpose.

Experimental Methods

All fundamental procedures and standardizations were carried out according to the methods outlined in Standard Methods of the New York State Department of Health, Wadsworth (25), for the complement-fixation test for tuberculosis. Preliminary tests were made in the presence of two units of complement; final tests by the method referred to above (Maltaner (14)). Twenty-four dilutions of complement ranging from a one in five or a one in ten to a one in one hundred were prepared in the same way as the complement dilutions for the daily complement titration. The final reaction in all tests was read by comparison with color standards prepared to detect differences of 5% or less in the degree of hemolysis.

(a) *Immune Serum*

Normal rabbits whose serum in 0.05-cc. amounts, after heating at 56° C. for one-half hour just previous to testing, did not show anticomplementary or nonspecific properties, were considered satisfactory for purposes of immunization. Before inoculation, large bleedings were taken and kept for use in parallel tests with immune serum from the same animal.

Some ten days after the animals had received the eighth intraperitoneal inoculation of vaccines prepared from killed cultures of tubercle bacilli, they were bled from the ear and the serum tested in the presence of standard S and R antigens. If good fixation was secured with 0.005 cc. or less of serum in the presence of 0.1 cc. of the optimum dilution of the standard antigens, the titre was considered satisfactory. If lower, the animal was given further inoculations.

(*b*) *Antigens*

Aqueous extracts of cultures of various strains were prepared according to the methods described in Standard Methods (25). Antigens which were still somewhat anticomplimentary in 0.02 cc. amounts after dialysis for one week, were either discarded or submitted to further dialysis.

A high titre antigen prepared from a virulent human S strain No. 13, Reed and Rice (20), which compared favorably with a carefully tested antigen made from the same strain, kindly supplied by Mrs. Maltaner, was selected as a standard S antigen. A highly active antigen prepared by a similar method from the typical R culture, No. 599, Reed and Rice (19), was chosen as a standard R antigen. The most sensitive dilutions of both of these antigens were determined by titrating in the presence of both S and R antisera, a high titre antituberculous horse serum, furnished by Mrs. Maltaner, and a No. 599 rabbit antiserum of high titre and low nonspecific activity. All new antigens were compared in parallel with the standard antigens in the presence of these two antisera.

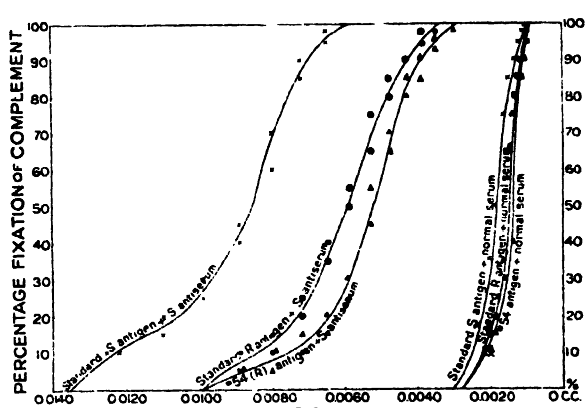


FIG. 1. *Results of a typical series of titrations of one immune and one normal serum in the presence of three different antigens (percentages inhibition of hemolysis plotted as ordinates; amount of complement fixed, as abscissas).*

(*c*) *Final Comparative Tests*

Each antigen, in 0.1 cc. amounts of the dilution found to be optimum, was tested in duplicate in the presence of various immune sera. If the serum was of high titre the serum volume was made up to 0.005 cc. by the addition of normal serum from the same animal species. Controls of the same antigen and 0.005 cc. of normal serum accompanied all tests.

The percentage inhibition of hemolysis at each dilution of complement was recorded graphically, the amounts of complement as ordinates, the percentages fixation as abscissas, Fig. I. Although the ratio between different systems was practically the same at 40, 50 and 60% hemolysis, all three points have been considered in the tables; at the extremes of 5 and 90% fixation the ratios

showed considerable variation. The specific fixation at any percentage inhibition of hemolysis was considered as the total amount of complement fixed by any one antigen in the presence of a particular antiserum less the amount of complement fixed in the presence of the same antigen and normal serum.

The standard deviation and probable error of a series of experiments carried out on the same day with the same antigen and serum, provided a measure of the accuracy of the methods. The probable error was always less than 10% with an average of 6.4%. Determinations which showed a greater variation between duplicates than this were disregarded. Similar calculations for the same series carried out on six different days with six different samples of pooled complement showed, as would be expected, a greater probable error because of the further variables introduced. The maximum probable error was 17.3%, the minimum 4.3% and the average 10.9%; the greatest percentage error was of course in the normal serum systems for here the variations bulk proportionately larger in view of the lesser total amount of complement fixed. The specific fixation is subject to the least degree of error because it is least dependent upon the quality of the complement.

Experimental Results

(a) Preliminary Studies with Standard S and R Antigens and Antisera

The preliminary studies of the antigenic activity of the S and R types of tubercle bacilli have been carried out with three strains of this organism: No. 599, an avirulent bovine strain; No. 54, an avirulent; and No. 13, a virulent human strain. The history and characteristics of these cultures were given in the second paper in this series (20). It was shown that No. 599 and No. 54 produce the typical R type of colony, form massive pellicles on liquid medium, agglutinate at relatively high acidity and show lack of virulence for guinea pigs: whereas, No. 13 produces the S type of colony and a veil-like pellicle on liquid medium, agglutinates at a relatively high acidity and exhibits virulence for guinea pigs.

In Table I are reported the results of a typical series of experiments carried out upon one day. Three antigens were compared in the presence of one immune serum and one normal rabbit serum, the degree of fixation being about the same in each case. The values have been plotted in Fig. I. A somewhat S-shaped curve similar to that reported by others who have studied the hemolytic reaction quantitatively (Brooks (7), Coulter (9) and others) was obtained.

The total fixation for S antigen No. 13 in the presence of R No. 599 antiserum at 40, 50 and 60% inhibition of hemolysis was 0.0080, 0.0072 and 0.0061 cc.; for R antigen No. 54, 0.0072, 0.0065 and 0.0055 cc.; and for antigen No. 599, 0.0078, 0.0069 and 0.0058 cc. The specific fixation, *i.e.*, the total fixation at these points less the amount of complement fixed in the normal system at the same points, was for the same antigens, 0.0063, 0.0057 and 0.0048; 0.0053, 0.0050 and 0.0043; 0.0060, 0.0052 and 0.0043 cc. respectively. If we compare the specific activity of the heterologous antigens at these three points with that of the homologous No. 599 antigen, we obtain these average ratios; for

the No. 13, an average of 1.08 to 1; for the No. 54 antigen the relationship is very similar, 0.95 to 1. The three antigens were apparently reacting with the same type of antibody in the antiserum, indicating some common antigenic property in S and R extracts. Each was apparently being used in sufficient excess to react with all the antibody present.

TABLE I

A COMPARISON OF THE PERCENTAGE INHIBITION OF HEMOLYSIS OBTAINED WITH ANTIGENS No. 13 (S), No. 54 (R) AND No. 599 (R) IN THE PRESENCE OF NORMAL AND IMMUNE RABBIT SERUM

Amount of complement cc.	Normal serum						Immune serum (No. 599)					
	No. 13 Antigen		No. 54 Antigen		No. 599 Antigen		No. 13 Antigen		No. 54 Antigen		No. 599 Antigen	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
0.0131	0	0	0	0	0	0	0	0	0	0	0	0
0.0118	0	0	0	0	0	0	10	5	0	0	10	10
0.0106	0	0	0	0	0	0	20	15	10	5	20	15
0.0096	0	0	0	0	0	0	30	30	15	15	30	25
0.0084	0	0	0	0	0	0	35	40	25	30	35	35
0.0076	0	0	0	0	0	0	40	50	30	40	40	50
0.0068	0	0	0	0	0	0	50	55	40	50	45	55
0.0061	0	0	0	0	0	0	60	60	50	60	50	60
0.0055	0	0	0	0	0	0	63	68	55	65	60	68
0.0050	0	0	0	0	0	0	70	75	60	70	65	70
0.0045	0	0	0	0	0	0	80	80	65	80	70	73
0.0040	0	0	0	0	0	0	90	90	70	83	73	78
0.0036	0	0	0	0	0	0	93	93	80	85	85	80
0.0032	0	0	0	0	0	0	95	98	85	93	93	90
0.0029	0	0	0	0	0	0	100	100	93	95	95	93
0.0026	0	5	10	5	5	5	100	100	95	100	98	98
0.0023	10	10	15	15	15	15	100	100	98	100	100	100
0.0021	15	25	30	25	25	20	100	100	100	100	100	100
0.0019	25	30	40	40	40	35	100	100	100	100	100	100
0.0017	40	40	45	45	50	50	100	100	100	100	100	100
0.0015	50	50	50	50	65	60	100	100	100	100	100	100
0.0013	60	60	55	60	70	70	100	100	100	100	100	100
0.0012	70	75	60	75	80	75	100	100	100	100	100	100
0.0011	80	80	75	80	90	80	100	100	100	100	100	100
0.0010	95	98	85	90	95	95	100	100	100	100	100	100

The results of a series of such tests have been assembled in Table II, the probable error of each mean value being inserted at the foot of the table. It may be seen, as was pointed out previously, that the per cent probable error on the specific fixation values is considerably less than that on the mean total amount of complement fixed. For this reason, in later experiments, specific fixation values only have been considered.

In Table III the results of similar series with different antigens and antisera have been averaged, each value representing the mean amount of complement fixed on five or more different days. The probable error of this mean value was calculated and has been inserted in the table below the mean values themselves. Although the slight differences in the amount of fixation by the various

TABLE II

Total and specific fixation of complement by an "R" bovine antigen No. 599 in the presence of its homologous immune serum

Date tested 1930	Total fixation of complement at			Specific fixation of complement at		
	40%	50%	60%	40%	50%	60%
May 7	0.0068 0.0072 0.0068	0.0066 0.0065 0.0065	0.0055 0.0060	0.0051	0.0047	0.0047
May 9	0.0076 0.0083 0.0078	0.0064 0.0070 0.0069	0.0061 0.0065	0.0054	0.0048	0.0046
May 14	0.0071 0.0071	0.0063 0.0065	0.0052 0.0058	0.0053	0.0053	0.0038
May 22	0.0080 0.0083	0.0076 0.0080	0.0064 0.0076	0.0054	0.0052	0.0050
May 29	0.0071 0.0072	0.0063 0.0063	0.0055 0.0054	0.0055	0.0053	0.0050
Average	0.0073	0.0068	0.0059	0.0053	0.0049	0.0047
Probable error	0.00079	0.00076	0.00067	0.0009	0.00028	0.00020

TABLE III

A comparison of the antigenic activity of antigens No. 13, 54 and 599 in the presence of No. 13, 54 and 599 antisera

Antiserum No.	Antigen No.	Total fixation at			Specific fixation at		
		40%	50%	60%	40%	50%	60%
13 (S)	13 (S)	0.0113 ±0.0014	0.0104 ±0.0014	0.0095 ±0.0013	0.0089 ±0.0003	0.0083 ±0.0006	0.0074 ±0.0005
13 (S)	54 (R)	0.0063 ±0.0006	0.0053 ±0.0003	0.0046 ±0.0002	0.0038 ±0.0004	0.0032 ±0.0004	0.0030 ±0.0004
13 (S)	599 (R)	0.0060 ±0.0007	0.0048 ±0.0005	0.0044 ±0.0005	0.0036 ±0.0004	0.0033 ±0.0004	0.0031 ±0.0004
54 (R)	13 (S)	0.0079 ±0.0006	0.0068 ±0.0006	0.0062 ±0.0004	0.0054 ±0.0005	0.0046 ±0.0004	0.0044 ±0.0004
54 (R)	54 (R)	0.0058 ±0.0006	0.0053 ±0.0004	0.0048 ±0.0005	0.0034 ±0.0003	0.0033 ±0.0002	0.0030 ±0.0002
54 (R)	599 (R)	0.0064 ±0.0009	0.0058 ±0.0008	0.0053 ±0.0007	0.0038 ±0.0003	0.0035 ±0.0004	0.0032 ±0.0004
599 (R)	13 (S)	0.0074 ±0.0013	0.0065 ±0.0007	0.0039 ±0.0006	0.0048 ±0.0008	0.0045 ±0.0006	0.0041 ±0.0005
599 (R)	54 (R)	0.0066 ±0.0007	0.0061 ±0.0007	0.0056 ±0.0007	0.0038 ±0.0002	0.0035 ±0.0002	0.0034 ±0.0003
599 (R)	599 (R)	0.0068 ±0.0005	0.0061 ±0.0004	0.0054 ±0.0003	0.0043 ±0.0001	0.0038 ±0.0003	0.0037 ±0.0002

antigens in the presence of the No. 599 and No. 54 antisera fell within the range of the probable error, in the presence of No. 13 antiserum, the No. 13 antigen fixed about twice as much complement as the R antigens, a difference equivalent to more than four times the probable error on the total fixation and at least eight times that on the specific fixation. In the presence of the standard S No. 13 antiserum, practically the same amount of fixation was secured with a 1 in 30 dilution of either antigen No. 54 or No. 599 as was given in a 1 in 20. In a dilution of 1 in 40 the fixation was decreased slightly. Antigen No. 599 diluted 1 in 30 and No. 54 diluted 1 in 25 contained sufficient antigenic substance to fix specifically about twice the amount of complement in the presence of two units of S antiserum that they fixed in the presence of one unit. The differences in the behavior of the R antigens would appear therefore to be of a qualitative rather than quantitative nature.

TABLE IV

RATIO OF THE SPECIFIC FIXATION OF COMPLEMENT BY ANTIGENS NO. 599, 54 AND 13 IN THE PRESENCE OF NO. 13, 54 AND 599 ANTISERA, TO THE SPECIFIC FIXATION IN THE PRESENCE OF THE NO. 13 ANTIGEN IN THE PRESENCE OF THE SAME ANTISERUM

Ratio at	Antiserum No.	Antigen No. 13 (S)	Antigen No. 54 (R)	Antigen No. 599 (R)
40% Inhibition of hemolysis	13 (S)	1:1	0.43:1	0.40:1
50% Inhibition of hemolysis	13 (S)	1:1	0.40:1	0.40:1
60% Inhibition of hemolysis	13 (S)	1:1	0.41:1	0.42:1
40% Inhibition of hemolysis	54 (R)	1:1	0.63:1	0.71:1
50% Inhibition of hemolysis	54 (R)	1:1	0.72:1	0.76:1
60% Inhibition of hemolysis	54 (R)	1:1	0.69:1	0.73:1
40% Inhibition of hemolysis	599 (R)	1:1	0 79:1	0.88:1
50% Inhibition of hemolysis	599 (R)	1:1	0.78:1	0.85:1
60% Inhibition of hemolysis	599 (R)	1:1	0.82:1	0.90:1

The similarity in the behavior of the S and R antigens in the presence of R antiserum, and their differences in the presence of S antiserum, is more apparent however from the ratios shown in Table IV.

(*b*) *Study of other Extracts and Antisera Prepared from the Same Strains*

These preliminary experiments seemed to show that extracts made from R organisms, whether of bovine or human origin, lacked something present in S preparations. Antisera prepared against S organisms contained not only those antibodies which reacted with R preparations, but also other antibodies not detected by R antigens, which reacted with an S antigen. The antibodies in the R antisera on the other hand, had their counterpart in both S and R antigens. The importance of using an S antigen in the titration of S antisera was therefore evident.

Extracts subsequently prepared from these same R strains behaved in the presence of the same antisera in a manner essentially similar to the first preparations. Certain minor quantitative or even qualitative differences between different preparations were to be expected; differences dependent upon the efficiency of extraction, the colloidal dispersion of the antigenic materials and the efficiency of the dialysis. As is shown in Table V, in the presence of two new S antisera, No. 6803 and 6804 respectively, the various R antigens were approximately one-third and one-half as efficient as the S antigens. These antisera would also seem to contain two types of antibodies, one of which is S-specific.

TABLE V

COMPARISON OF THE ACTIVITY OF AN S AND VARIOUS R ANTIGENS IN THE PRESENCE OF TWO S ANTISERA, USING THE SPECIFIC FIXATION THE STANDARD S (13) ANTIGEN AS THE UNIT OF COMPARISON

Antigen No.	Antiserum (S) No.	Specific fixation at			Ratio of specific fixation to that with the standard S antigen		
		40%	50%	60%	40%	50%	60%
Standard S	6803	0.0137	0.0116	0.0111	1:1	1:1	1:1
599–1–R	6803	0.0053	0.0049	0.0046	0.39:1	0.42:1	0.41:1
599–2–R	6803	0.0053	0.0047	0.0044	0.39:1	0.41:1	0.39:1
599–3–R	6803	0.0038	0.0037	0.0035	0.27:1	0.31:1	0.31:1
54–1–R	6803	0.0033	0.0028	0.0026	0.27:1	0.25:1	0.23:1
54–2–R	6803	0.0046	0.0040	0.0039	0.33:1	0.34:1	0.35:1
Standard S	6804	0.0060	0.0058	0.0056	1:1	1:1	1:1
599–1–R	6804	0.0038	0.0036	0.0032	0.53:1	0.62:1	0.57:1
599–2–R	6804	0.0033	0.0028	0.0022	0.55:1	0.49:1	0.40:1
599–3–R	6804	0.0038	0.0027	0.0023	0.63:1	0.48:1	0.41:1
54–1–R	6804	0.0040	0.0032	0.0029	0.67:1	0.55:1	0.52:1
54–2–R	6804	0.0032	0.0027	0.0022	0.54:1	0.46:1	0.41:1

(c) *Behavior of Antigens Prepared from other Cultures of Mammalian Tubercle Bacilli*

Other known S and R cultures of tubercle bacilli were used in the preparation of antigens and antisera. An aqueous extract of the virulent bovine strain, No. 56, previously studied (20), was found to behave in the presence of R antiserum and with both the standard S No. 13 and its own homologous antiserum, in a manner similar to the S antigens previously studied (Table VI). The No. 56 antisera also contained antibodies with which R antigens, whether of human or bovine origin, did not react.

Several strains of B.C.G. have been maintained in this laboratory for some time. One of these, the Paris strain, has for two years consistently produced only R colonies and has failed to produce progressive tuberculosis in guinea pigs. Another, the Perif. strain isolated from a culture of B.C.G. obtained from Dr. Petroff, is composed of almost pure S types and leads in guinea pigs to a generalized tuberculosis. Aqueous extracts and antisera were prepared from both of these strains.

In the presence of standard R antiserum, the two B.C.G. antigens were only some 40 to 50% as efficient as the standard S and R antigens (Table VI). The reason for this is probably an unsatisfactory extraction since the extracts were water clear, unless indeed B.C.G. cultures are low in antigenic properties. Notwithstanding the relative inefficiency of antigens, there appeared to be a significant difference in the behavior of the Perif or S antigens and the Paris or R antigens, in the presence of the standard S antiserum, the former being

TABLE VI

A COMPARISON OF THE ACTIVITY OF VARIOUS S AND R ANTIGENS IN THE PRESENCE OF S AND R ANTISERA WITH THAT OF THE STANDARD S AND R ANTIGENS

Antigen	Specific fixation at			Ratio of specific fixation to that of	
	40%	50%	60%	Standard R	Standard S
(a) 599 Antiserum (R)					
56-1	0.0078	0.0077	0.0076	0.80:1	0.97:1
B.C.G.-Perif.	0.0050	0.0046	0.0041	0.48:1	0.58:1
B.C.G.-Paris	0.0049	0.0039	0.0030	0.41:1	0.50:1
(b) 56 Antiserum (S)					
56-1	0.0080	0.0079	0.0077	1.49:1	1.02:1
54-1	0.0050	0.0047	0.0046	0.98:1	0.62:1
B.C.G.-Paris	0.0030	0.0027	0.0025	0.52:1	0.36:1
B.C.G.-Perif.	0.0047	0.0045	0.0044	1.11:1	0.77:1
(c) B.C.G.-Paris antiserum (R)					
13-2	0.0078	0.0076	0.0070	0.93:1	0.81:1
56-2	0.0073	0.0071	0.0060	0.84:1	0.73:1
B.C.G.-Paris	0.0098	0.0090	0.0088	1.16:1	0.89:1
B.C.G.-Perif.	0.0088	0.0087	0.0073	1.02:1	0.89:1
(d) B.C.G.-Perif. antiserum (S)					
B.C.G.-Perif.	0.0051	0.0044	0.0040	1.41:1	0.67:1
B.C.G.-Paris	0.0019	0.0017	0.0016	0.56:1	0.26:1

nearly twice as active as the latter. Whereas, in the presence of R antiserum, the efficiency ratio of the Perif antigen to the standard R antigen was 0.48 to 1, in the presence of the S antiserum, the ratio became 1.11 to 1, a difference made up doubtless by the presence of certain S antigenic substances in the Perif. preparations. Such a supposition receives further confirmation in the observation that the efficiency ratios of this antigen to the standard S and R antigens were also increased in the presence of S antisera. The Paris antigen behaves in all respects like other R antigens of low titre. These findings, although far from complete, suggest that the change in colony form and acquisition of virulence in this one strain of B.C.G. Perif has been accompanied by a gain in specific antigenic substances. The undissociated strain of B.C.G. appears to resemble other R strains in antigenic characteristics.

As may be seen from Table VII, these B.C.G. strains produce antisera which react in a manner similar to antisera prepared against other S and R tubercle bacilli when tested with standard S and R antigens. The results of tests of various additional S and R antisera with these standard antigens further support the conclusions previously reached.

(*d*) *Antigenic Relationship with S and R Types of Other Acid-fast Species*

Several stains of other acid-fast species have been dissociated (18). Comparative tests were made with one strain of *Myco. leprae*, the L-65 or Duval strain obtained from the American Type Culture Collection, from which very definite S and R types had been isolated, and with Petroff's avian S and R cultures (17, 20).

TABLE VII

A COMPARISON OF THE REACTIVITY OF THE STANDARD R AND S ANTIGENS IN THE PRESENCE OF VARIOUS ANTISERA

Antiserum	Antigen	Specific fixation at 40%	Specific fixation at 50%	Specific fixation at 60%	Average ratio S:R	Average ratio R:S
(a) S antiserum						
6805 (56)	St. S	0.0089	0.0075	0.0066	1.46:1	—
6805 (56)	St. R	0.0056	0.0052	0 0050	—	0.67:1
956 (13)	St. S	0.0059	0.0053	0.0048	1.77:1	—
956 (13)	St. R	0.0031	0.0030	0.0029	—	0.56:1
986 (13)	St. S	0.0050	0.0045	0.0044	1.98:1	—
986 (13)	St. R	0.0025	0.0023	0.0022	—	0.50:1
B.C.G.-Perif.	St. S	0.0071	0.0064	0.0062	2.05:1	—
B.C.G.-Perif.	St. R	0.0039	0.0030	0.0027	—	0.50:1
6803 (13)	St. S	0.0060	0.0038	0.0056	1.41:1	—
6803 (13)	St. R	0.0038	0.0032	0.0032	—	0.60:1
(b) R antiserum						
6801 (599)	St. S	0.0080	0.0079	0.0078	0.82:1	—
6801 (599)	St. R	0.0100	0.0099	0.0090	—	1.22:1
958 (599)	St. S	0.0093	0.0092	0.0088	0.97:1	—
958 (599)	St. R	0.0095	0.0094	0.0093	—	1.03:1
980 (599)	St. S	0.0098	0.0093	0.0088	0.78:1	—
980 (599)	St. R	0.0132	0.0121	0.0108	—	1.26:1
970 (54)	St. S	0.0070	0.0068	0.0067	0.76:1	—
970 (54)	St. R	0.0096	0.0090	0.0083	—	1.33:1
B.C.G.-Paris	St. S	0.0099	0.0090	0.0087	1.16:1	—
B.C.G.-Paris	St. R	0.0085	0.0080	0.0077	—	0.87:1

Extracts of the S and R leprae cultures, after preliminary tests, were compared with the standard S and R tubercle bacillus antigens in the presence of various S and R tuberculous antisera (Table VIII). The S leprae extracts were always definitely inferior to the R extracts of the same organisms. Part at least of this difference appeared to be due to incomplete extraction, although the possibility that these S organisms contained less of the antigenic substances common to the two species of acid-fast bacteria than the R types of the same, could not be disregarded.

The data in Table VIII would also seem to indicate that antisera prepared against the R type of tubercle bacillus contained a higher percentage of antibodies reacting with the related acid-fast species, *Myco. leprae*, than antisera prepared against the S types of tubercle bacilli.

Aqueous extracts made from Petroff's avian S and R cultures, when tested in the presence of various S and R human and bovine tuberculous antisera, were found to behave in a very similar manner to the leprae antigens just described, only a fraction of the antibodies contained in these sera being fixed. That this fixation was due, in part at least, to the presence of group antibody was shown by the fact that they also reacted to some extent with the leprae antisera. The avian R antigens reacted somewhat more strongly than the S with the tuberculous antisera, another finding which is in agreement with the observations made with leprae antigens.

TABLE VIII

A COMPARISON OF THE AVERAGE SPECIFIC FIXATION OF ANTIGENS PREPARED FROM S AND R CULTURES OF LEPRAE BACILLI IN THE PRESENCE OF VARIOUS ANTISERA, WITH THE SPECIFIC FIXATION OF THE STANDARD S AND R TUBERCLE BACILLUS ANTIGENS IN THE SAME SYSTEM

Antiserum	Leprae S: St. S	Leprae S: St. S	Leprae R: St. S	Lepare R: St. S
(a) S antisera				
100 (13)	0.13:1	0.34:1	0.35:1	0.69:1
6804 (56)	0.23:1	0.35:1	0.33:1	0.48:1
6803 (13)	0.29:1	0.49:1	0.30:1	0 43:1
956 (13)	0.22:1	0.42:1	0.39:1	0.65:1
983 (13)	0.21:1	0.43:1	0.42:1	0.76:1
B.C.G.–Perif.	0.17:1	0.38:1	0.27:1	0.58:1
Average	0.20:1	0.40:1	0.34:1	0.60:1
(b) R antisera				
6801 (599)	0.32:1	0.39:1	0.65:1	0.52:1
980 (599)	0.31:1	0.24:1	0.62:1	0.48:1
958 (599)	0.14:1	0.11:1	0.46:1	0.44:1
970 (500)	0.05:1	0.04:1	0.53:1	0.41:1
Average	0.21:1	0 19:1	0.57:1	0.46:1

(e) *Suggestions as to the Type of Antibodies Constituting S and R Antisera*

In Table IX are summarized some of the data from the preceding tables and also data from several experiments not here recorded. The tuberculous immune sera have been divided into two groups, those prepared against a virulent strain of tubercle bacillus showing typical S-like colonies, and those prepared against attenuated strains characterized by R-like colonies. These sera have been tested in the presence of various S and R antigens, but for conciseness, only the standard S and R antigens of the tubercle bacillus and of the R antigens of the leprosy bacillus are dealt with.

In an S antiserum, an S antigen present in some excess, may be considered to fix 100% of the antibodies; the other two antigens, various percentages of this amount as determined by the ratios previously shown. Similarly in an R antiserum, the standard R antigen would fix 100% of the antibodies, the other antigens different proportions of this total amount.

The existence in an S antiserum of three types of antibodies may be postulated: (a) those specific for the S type only; (b) those reacting also with the R type, a species antibody; and (c) those which react in addition with related acid-fast species, a group antibody. Corresponding to these antibodies, there would be present in the S organisms, the type-specific or S-specific, the species-specific and group antigenic substances.

TABLE IX

PERCENTAGE FIXATION WITH VARIOUS ANTIGENS IN THE PRESENCE OF VARIOUS R AND S TUBERCULOUS IMMUNE SERA AS COMPARED WITH THE SPECIFIC FIXATION WITH THE HOMOLOGOUS R OR S STANDARD ANTIGEN

Antisera	Per cent of antibodies fixed by			Antisera	Per cent of antibodies fixed by		
	Standard S	Standard R	Leprae R		Standard S	Standard R	Leprae R
(a) S (T.B.) antisera				(b) R (T.B.) antisera			
100	100	41	35	6801	82	100	52
6803	100	41	—	6802	114	100	—
6804	100	67	33	970	76	100	41
956	100	56	39	980	78	100	48
8866	100	50	62	958	97	100	44
Perif.	100	50	27	Paris	110	100	52
6805	100	60	48	5799	96	100	44
6806	100	57	36	6800	90	100	50
6807	100	47	46	Average	93	100	47
Average	100	52	41				

Using the values in Table IX, an average S antituberculous serum may be visualized as consisting of 48% S-specific, 11% species-specific and 31% group-specific antibodies. In referring to the S-specific antibodies as type-specific, it should be noted, as has been done in a previous section, that bovine and human stains seem to elicit the same type-specific antibodies, at least as denoted by the complement fixation reaction, but that these are different from the type-specific factors of the avian strains.

By similar reasoning, an average R antiserum would contain 7% R-specific, 46% species-specific and 47% group antibodies, giving about the same proportion of group antibodies in both S and R antisera. With the change from S to R, there has been a substitution of species-specific factors for the type-specific groups. Whether the 7% R-specific substances has any significance is doubtful because such a low value falls within the range of the probable error of the specific fixation values.

Discussion

Investigation of the antigenic properties of S and R types of various species has indicated that the S and R change in those species in which the S form is the normal one, involves a loss in specificity, the resultant R type being more closely related to the R of other strains than to its homologous S.

The change from S to R in tubercle bacilli also seems to involve a loss in some S-specific substance, an antigenic change of the type occurring in the dissociation of the pneumococci and streptococci, but whether this change is related to the loss of carbohydrate substance, as in the case of the pneumococcus has not been determined. In addition to the S-specific and species antigen, there also appears to be a group antigen in the tubercle bacillus, an antigen which reacts with antisera prepared against other acid-fast species. This reaction is reciprocal, antisera prepared with vaccines of either S or R types of tubercle bacilli containing antibodies which react with closely related acid-fast bacteria. That part of this group reaction may depend upon a non-specific reaction with certain lipoids contained in extracts of *Myco. leprae* or avian tubercle bacilli, which like all other acid-fast organisms are high in lipoidal content, is suggested tentatively.

The observation that R antigens detect only some 50% of the antibodies in sera prepared against virulent organisms, in fact that they are only a little better than extracts prepared from related acid-fast species, seems of importance from a diagnostic standpoint. It is just possible that some of the unsatisfactory results which have obtained with the complement-fixation test in the diagnosis of tuberculous infection, may have been due in part to the use of an antigen prepared from cultures which were not fully virulent. Although it is not known in how far tuberculous antibodies detected by the complement-fixation reaction are related to immunity in the infected animal, it does not seem unjustifiable to wonder in how far these differences in antigenic behavior may affect the value of R cultures as immunizing agents.

Summary

By a quantitative technique, it has been shown that the S to R change in human and bovine tubercle bacilli is accompanied by a loss in specific antigenic activity in the complement-fixation reaction. All preparations from attenuated cultures in which the R colony has been shown to be predominant, have been found to be much inferior as antigens to similar preparations made from cultures composed of S types.

Antisera prepared from virulent S organisms have been shown to contain S-specific antibodies, in addition to species antibodies which also react with R antigens, and group antibodies detected by preparations made from related acid-fast species. The S-specific antigenic substances in virulent human and virulent bovine cultures appeared to be qualitatively similar.

Antisera prepared against R organisms have been found to contain species and group antibodies, the former being present in a higher proportion than in S antisera.

Acknowledgment

The author is indebted to Dr. G. B. Reed of the Department of Bacteriology, Queen's University, and to Dr. W. L. Holman of the Department of Pathology and Bacteriology of the University of Toronto, for helpful suggestions and criticisms.

References

1. ARKWRIGHT, J. A. J. Path. Bact. 31: 665-670. 1928.
2. ARKWRIGHT, J. A. and PITT, R. M. J. Path. Bact. 32: 229-246. 1929.
3. ARONSON, J. D. and WHITNEY, C. E. J. Infectious Diseases, 47: 30-55. 1930.
4. BABÈS, B. and BUSILIA. Compt. rend. soc. biol. 68: 181. 1910.
5. BEGBIE, R. S. Edinburgh Med. J. 37: 187-191. 1930.
6. BORDET, J. and GENGOU, O. Compt. rend. soc. biol. 73: 122. 1903.
7. BROOKS, S. C. J. Med. Research, 41: 399. 1920.
8. COOKE, J. V. J. Infectious Diseases, 25: 452-473. 1919.
9 COULTER, C. B. J. Gen. Physiol. 10: 541-550. 1927.
10. COULTHARD, H. L. J. Path. Bact. 26: 350-381. 1923.
11. GRIFFITH, F. A system of bacteriology, v. 5: 150. 1930.
12. HARRIS, W. H. and LANFORD, J. A. J. Infectious Diseases, 13: 301-308. 1913.
13. KIRCHNER, O. Beit. Klin. Tuberk. 66: 584-587. 1927.
14. MALTANER, E. J. Immunol. 1931. (In press).
15. PETROFF, S. A., BRANCH, A. and STEENKEN, W. Proc. Soc. Exptl. Biol. Med. 25: 14-16. 1927.
16. PETROFF, S. A., BRANCH, A. and STEENKEN, W. Am. Rev. Tuberculosis, 19: 9-39. 1929.
17. PETROFF, S. A. and STEENKEN, W. J. Exptl. Med. 51: 831-845. 1930.
18. REED, G. B. J. Bacteriol. (In press).
19. REED, G. B. and RICE, C. E. Can. J. Research, 4: 389-398. 1931.
20. REED, G. B. and RICE, C. E. Can. J. Research, 5: 111-121. 1931.
21. SCHEFF, L. D. Am. Rev. Tuberculosis, 12: 45-48. 1925.
22. SHIBATA, J. Z. Immunitäts. 63: 358-370. 1929.
23. SMITH, J. Lancet, 203 (2): 13-14. 1922.
24. TODD, E. W. and LANCEFIELD, R. C. J. Exptl. Med. 48: 751-767. 1928.
25. WADSWORTH, A. B. Standard methods of the Division of Laboratories and Research of the New York Department of Health, Williams and Wilkins, Baltimore, 1927.
26. WADSWORTH, A., MALTANER, F. and MALTANER, E. J. Immunol. 10: 241-332. 1925.
27. WHITE, P. B. A system of bacteriology. v. 4. 1929.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL *of* CANADA

VOLUME 5 | OCTOBER, 1931 | NUMBER 4

PREPARATION AND HEAT DENATURATION OF THE GLUTEN PROTEINS[1]

BY W. H. COOK[2]

Abstract

Gliadin prepared by several different methods had the same nitrogen content and distribution. The critical peptization temperature (C.P.T.) in 60% alcohol and viscosity in 30% urea-buffer solutions, however, showed considerable variation, preparations of high C.P.T. (low solubility) being more viscous. This variation in the physical properties is explained by fractionation or denaturation incidental to the method of preparation.

Gluten precipitated from 30% urea solutions at salt concentrations varying from 0.1 to 0.5 of saturation, yielded fractions that varied continuously in their gliadin and glutenin content, as judged from their percentage of arginine nitrogen.

Gluten dispersed in buffered 30% urea solutions showed no change in viscosity during 101 hr. after the gluten was completely dispersed. A variation of hydrogen ion concentration between pH 6.0 and 6.95 had little effect on its viscosity. Heating at 70° C. caused a marked decrease in the viscosity of this dispersion during the first hour. When gliadin dispersions are heated as above only samples having a high initial viscosity and C.P.T. become less viscous. Heating gliadin of natural moisture content (12 to 14%) at 70° C. for varying periods of time did not change significantly its subsequent C.P.T. and viscosity in 60% alcohol. More severe heat treatments at higher moisture contents rendered the gliadin insoluble in 60% alcohol. Dilute alcoholic extracts of heated flours contained less protein than those of unheated controls. However, the C.P.T. of the former was lower than that of the latter. It is concluded from these experiments that when the gluten proteins are subjected to elevated temperatures, the glutenin fraction is first affected, next the gliadin fractions of low solubility, and finally, under severe conditions, all of the gliadin is denatured.

Introduction

The available data on the heat denaturation of the gluten proteins have been obtained largely from investigations conducted on either flour or gluten. This has been inevitable since the gliadin and glutenin, which make up gluten, have been difficult to isolate and study without altering their colloidal properties. Recent experiments have shown, however, that the changes induced in these

[1] *Manuscript received July 31, 1931.*

Contribution from the Food Research Institute and Department of Chemistry, Stanford University, California, and the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. The present paper is being issued as Paper No. 26 of the Associate Committee on Grain Research. It constitutes part of a thesis presented to the Department of Chemistry, Stanford University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] *Junior Research Biologist, National Research Laboratories of Canada.*

proteins by elevated temperatures are of commercial as well as theoretical importance, and this has stimulated research along these lines. The present investigation was conducted to determine how the two gluten proteins were affected by heat treatment.

The commercial value of heat-treated flour has been demonstrated recently by Kent-Jones (16) who found that the baking quality was improved by controlled heat treatment. Moreover, he has found that an excessively heat-treated flour, although ruined for bread making purposes when used alone, may be used in small amounts as a flour improver. Both Kent-Jones (16) and Herd (13) conclude that some alteration of the protein is responsible, not only for the decreased baking quality of the excessively heat-treated flour when used alone, but also for the increased baking quality when such a flour is added to other flours as an improver.

An extensive investigation on the drying of wheat, conducted by the National Research Council of Canada (17) showed that wheat decreases in quality when the temperature of the air used for drying exceeds 180° F. At higher air temperatures the decrease in quality is roughly proportional to the temperature increase.

Geddes (7, 8) has repeated part of the investigations carried out by Kent-Jones. Under certain conditions of heating an improvement in baking quality was observed, while more excessive heat treatments resulted in a corresponding decrease in baking quality. He found that the extent and rate of swelling of the gluten washed from these flours, and the viscosity of acidulated flour-in-water suspensions decreased with increasing severity of heat treatment. Geddes also determined the amount of protein dispersed by a normal solution of magnesium sulphate and potassium iodide by the method of Gortner, Hoffman and Sinclair (9), and found that the flour proteins became progressively less soluble with increasing amounts of heat treatment. He concluded that the effect of heat on the gluten proteins is always detrimental to baking quality, and that the improvement observed after mild heat treatments is probably due to the effect of heat on the lipoid fraction of the flour.

The temperature at which the gluten is altered depends on the moisture content and time of heating. Kent-Jones (16) found that it was necessary to heat flour of "natural moisture content" for 20 hr. at 130° F. (54.4° C.) to produce a change similar to that obtained by two hours' heating at 150° F. (65.6° C.). Geddes' results (7) show that six to eight hours' heating at 130° F. (54.5° C.) are required to produce a significant change in the baking quality of a flour containing 13.9% moisture. Geddes (7, 8) has shown that the effect of heat on flour, as judged by its subsequent baking quality and protein solubility, increases with its moisture content. This is in harmony with the earlier work of Chick and Martin (4), who found that moist egg albumin could be subjected to temperatures of 120° C. for several hours before any appreciable denaturation took place, provided the moisture was allowed to evaporate during the treatment. If, however, the protein were placed in a sealed tube (*i.e.*, in the presence of steam), complete denaturation occurred at this temperature in a few moments.

Alsberg and Griffing (1) found that the swelling of gluten disks, subjected to 30 min. heating at various temperatures from 50° to 80° C., decreased as the temperature increased. The rate of decrease in swelling power was greatest between 60° and 65° C., and with this exception these workers found no indication of a definite coagulation temperature. This appears to be in agreement with the work of Chick and Martin (4) on egg albumin; these writers do not regard coagulation as taking place at a definite temperature, but rather consider that this process is a chemical reaction with a very high temperature coefficient. At temperatures between 30° and 50° C., Alsberg and Griffing found no decrease in swelling power, in fact, they obtained some evidence of an increased swelling.

There is little information as to the effect of heat on the individual gluten proteins except that which has been inferred from indirect evidence. Purified glutenin has apparently never been studied from this standpoint, although it is known that a dispersion of glutenin in dilute alkali is not coagulated on heating, thus differing from egg albumin in its reaction to heat. Gottenberg and Alsberg (10) found that gliadin dispersed in 60% alcohol was not seriously altered in solubility when held at a temperature of 58° C. for 15 days. This indicates that either it is not affected by being heated to this temperature, or that the 60% alcohol protected it from denaturation. This latter possibility is suggested by the fact that gliadin dispersed in 20% alcohol showed a significant increase in its C.P.T. on being heated.

Experimental

Methods of Analysis

The methods employed for determining the moisture and nitrogen contents and the nitrogen distribution were the same as those described in an earlier paper by Cook and Alsberg (5).

Hydrogen ion concentration. This determination was made with a quinhydrone electrode at 25° C.

Viscosity. All viscosity measurements were made in Ostwald viscometers. These were calibrated with water and sucrose solutions, using the values given in the International Critical Tables (15, p. 23). The measurements were made in a thermostat at 25° C. ±0.05, the specific gravity determinations being carried out at the same temperature.

Critical peptization temperature. This has been defined by Dill and Alsberg (6) as the temperature at which turbidity appears when a solution of gliadin in alcohol-water is cooled. On the other hand, if the gliadin is originally turbid in dilute alcohol at ordinary temperatures, this turbidity will usually clear up at a definite temperature which can be observed if the dispersion is heated slowly. The temperature at which the solutions became clear on heating, and the temperature at which they again became turbid on cooling were averaged to obtain the reported values. The technique employed was that described by Dill and Alsberg.

Preparation and Properties of Gliadin

Before studying the heat denaturation of gliadin, some attention was given to the preparation of this protein. Three methods described in the literature were employed in preparing samples. One of these methods yielded three fractions. Another preparation was made using urea solution as the dispersion medium. A description of these preparations follows. Only the procedure which yielded different fractions, and the urea method, are described in detail.

Preparation 1. This sample of gliadin was prepared by extraction with 70% alcohol in essentially the same manner as that described by Osborne (18).

Preparation 2. This sample was prepared by extraction with 60% alcohol and purified by the method of Dill and Alsberg (6). This procedure is essentially that of Osborne, using additional precautions to obtain purity and prevent denaturation.

Preparations 3 and 4. These preparations were obtained from an acetic-acid extract of dried gluten, using the method of Blish and Sandstedt (2). The 18 litres of 0.07 *N* acetic acid, containing the dispersed gliadin from the initial extraction, was filtered through paper pulp and 200 gm. of lithium chloride added to precipitate the gliadin. The solution was stirred vigorously while adding the salt, in order to precipitate the gliadin as a foam which is easy to wash. The foam was then removed and allowed to stand at 2.5° C. overnight and the drainage water removed. It was then adjusted to a 60% alcohol concentration and again allowed to stand at 2.5° C., which gave a precipitate and a clear supernatant liquid, which was concentrated at low pressure and below 50° C. to about one-fourth its original volume. On standing at 2.5° C., this solution did not give a precipitate, so it was precipitated by pouring it into 1% lithium chloride solution and purified separately. On purification by one aqueous and one alcoholic precipitation, this gave Preparation 3. The precipitate obtained at 2.5° C. was unfortunately lost by accident in dehydrating the material. Preparation 4 was obtained from the original acetic acid dispersion. After removing the foam, this liquid was quite turbid, so it was allowed to stand overnight at 0° C. The resulting precipitate did not give a clear solution in 60% alcohol, and only a small amount of material settled out on standing. After discarding this precipitate, the dispersion, though opaque, appeared to be quite homogeneous at room temperature. It was consequently purified by one aqueous and one alcoholic precipitation, and dehydrated.

Preparation 5. This preparation was obtained by dispersing freshly washed gluten in 30% urea, supercentrifuging to remove the starch, and then precipitating the glutenin at 0.18 of saturation with magnesium sulphate. The precipitated glutenin was removed by supercentrifuging, and 500 gm. of solid magnesium sulphate added to two litres of the liquid so obtained. The clear liquid immediately became milky but as no precipitate settled out on standing 24 hr. at 0° C., it was removed by supercentrifuging. The liquid which was removed still contained considerable protein, but it was discarded. Since gliadin is dispersed by a 10% urea solution (5), the precipitate from the supercentrifuge was placed in a urea solution of this concentration in which it gave

an opaque dispersion. Addition of magnesium sulphate to 0.12 of saturation precipitated most of the protein. On redispersing the protein in 10% urea, and supercentrifuging to remove the denatured material, a clear solution was obtained. This was poured into five volumes of water and allowed to stand 24 hr. to settle. The precipitate was then washed and dehydrated with alcohol and ether. This preparation was dispersed by 60% alcohol, but the dispersion was more opaque than that of gliadin prepared by the alcohol method.

The nitrogen content and distribution of these preparations are given in Table I. Preparations 1, 2, 3 and 5, isolated by different methods, appear to have essentially the same composition. Preparation 4 has a lower total nitrogen content, and a higher percentage of amide nitrogen, for which there is no apparent explanation. This preparation also has a significantly lower arginine content. Owing to the peculiar solubility of this sample, and its difference in composition, it was not included in the average.

TABLE I

NITROGEN CONTENT AND DISTRIBUTION OF GLIADIN PREPARATIONS

			Nitrogen distribution								
						Phosphotungstic acid precipitation					
							Values for fractions corrected for arginine nitrogen in filtrate				
Preparation No.	Method of preparation	Nitrogen content	Humin N	Amide N	Arginine N	Arginine N in filtrate	Basic N	Total filtrate N	Filtrate amino N	Filtrate non-amino N	Recovery
		%	%	%	%	%	%	%	%	%	%
1	Osborne (18)	17.30	0.006	25.4	5.67	3.21	10.4	62.7	54.4	8.3	98.5
2	Dill and Alsberg (6)	17.48	0.001	26.0	5.81	3.31	12.0	62.0	55.0	7.0	100.0
3	Blish and Sandstedt (2)	17.71	0.007	26.1	5.76	2.88	9.4	62.8	53.3	9.5	98.3
4	Blish and Sandstedt (2)	17.24	0.001	28.0	5.19	2.64	10.0	61.6	53.3	8.3	99.6
5	Urea dispersion	17.57	0.040	26.6	5.80	3.20	10.8	63.6	56.1	7.5	101.0
Average of preparations No. 1, 2, 3 and 5		17.50	—	26.0	5.76	—	10.6	62.8	54.7	8.1	—

The physical properties of these preparations, however, are quite different. Their critical peptization temperatures, (C.P.T.), viscosities of 5% dispersions in 60% alcohol (by volume), and viscosities of 4% dispersions in buffered 30% urea solutions are given in Table II.

In this table the gliadin samples have been arranged in the order of increasing C.P.T. It was impossible to get a sharp reading for the C.P.T. of Preparations 4 and 5, but the dispersions did clear up in the region of the reported temperatures. It is evident that there is a relationship between the C.P.T. and the viscosity of the dispersions, those of high C.P.T. being more viscous.

TABLE II

CRITICAL PEPTIZATION TEMPERATURE AND VISCOSITY OF GLIADIN PREPARATIONS

Preparation No.	Method of preparation	C.P.T. °C.	Gliadin (5%) in 60% alcohol, centipoises	Gliadin (4%) in 30% urea-buffer, centipoises
3	Blish and Sandstedt	−7.4*	—	2.64
1	Osborne	7.6	5.30	2.59
2	Dill and Alsberg	12.4	5.90	3.05
5	Urea-dispersion	35–40**	—	3.24
4	Blish and Sandstedt	80–90**	—	5.91

**This low C.P.T. can be attributed partly to some acetic acid having been retained by the protein (See Table VIII).*

***No definite temperature.*

These results show that although different samples of gliadin prepared from the same flour, by various methods, have the same chemical composition, they differ considerably in their physical properties. This can be explained either by a fractionation of the original material, or by a partial denaturation of the gliadin.

The first explanation appears to be the correct one, judging from the recent work of Haugaard and Johnson (12). These workers have shown that gliadin can be divided into a number of fractions differing in solubility, although almost the same in chemical composition. They have also shown that each portion so obtained can be fractionated further, again yielding one portion of lower and another of higher solubility than the original. This provides an explanation for the low C.P.T. of Preparation 3 and the high C.P.T. of Preparation 4 which were both obtained from the same gluten extract. It also explains the relatively high C.P.T. of the preparation from urea, for only part of the gliadin was precipitated from the supernatant liquid, and presumably this represented the more insoluble fractions. Haugaard and Johnson also found that fractions of low maximum solubility had higher viscosities and C.P.T.'s than those of high maximum solubility, a finding in agreement with the above results.

The possibility of some denaturation having taken place in these gliadin preparations cannot be entirely excluded. Although the different physical properties can be explained by the extraction of different fractions, it was found that these samples all gave a positive test for a thiol group when tested with sodium nitroprusside. The work of Harris (11) and Hopkins (14) has shown that with such proteins as egg albumin this reaction can be taken as an index of denaturation. It may be, however, that gliadin gives this reaction originally although it seems probable that it does not (5). The urea preparation showed no significant difference from the others in this respect.

FRACTIONATION OF GLUTEN

Glutenin is a more difficult protein to study than gliadin from the standpoint of heat denaturation. Although a method has been developed for

preparing this protein in neutral solution, it has so far been found impossible to obtain glutenin in a dry form without denaturing it. A study of the heat denaturation of glutenin must therefore be confined to dispersions.

The possibility of glutenin not being a definite entity complicates its study still further. Blish and Sandstedt (3) conclude that there is insufficient evidence to show that glutenin is a chemical individual. The similar composition of different glutenin samples prepared from the same flour by the urea method (5) makes the possibility of different chemical fractions seem unlikely. Glutenin may, however, be separable like gliadin into a number of fractions which have different physical properties, though almost identical chemically. Although no definite evidence of this has yet been obtained it was observed in preparing glutenin by the urea method that this protein was not completely precipitated, as judged by the opaque supernatant fluid, either by the addition of salt or water, until sufficient had been added to come just within the lower precipitation limit for gliadin. Furthermore, the precipitation of gluten from a urea dispersion appeared to be a continuous process, for after removing the first precipitate, a further addition of salt or water would yield another precipitate. To obtain further information on this point a sample of gluten was fractionated in the following manner:

A dispersion of gluten was prepared by placing 200 gm. of wet gluten in 1500 cc. of 30% urea solution at pH 6.9. After standing overnight the dispersion was supercentrifuged to remove the starch. Saturated magnesium sulphate in 30% urea solution was then added until the dispersion had a salt concentration of 0.13 of saturation. This was allowed to stand 1½ hr., supercentrifuged to remove the precipitated protein, and the salt concentration of the liquid adjusted to 0.16 of saturation. The process was then repeated, and fractions obtained at salt concentrations corresponding to 0.13, 0.16, 0.18, 0.21 and 0.27 of saturation. Solid magnesium sulphate was then added to the solution until it was approximately half saturated. This made the liquid turbid, but some difficulty was encountered in getting this material to settle. Finally, it was accomplished by adjusting the hydrogen ion concentration to about pH 5.5 by the addition of a little dilute acetic acid. The complete solubility of this fraction in 60% alcohol proved it to be gliadin, so no further effort was made to recover the protein which remained dispersed. As the further purification of these samples had to be accomplished without dispersing them, since this was found in an earlier experiment to result in a further fractionation, a uniform procedure was employed throughout. All the fractions were washed eight times with ice-cold 1% lithium chloride solution. This removed most of the urea and yet prevented the partial dispersion of the more soluble fractions. These washings, however, were not very effective, as the precipitates came down in a very coherent form and had to be torn up several times during these extractions. The samples were then treated with 95% alcohol, ground up in a mortar, dehydrated with absolute alcohol and ether, and finally dried to constant weight. The nitrogen content and distribution of these fractions are given in Table III.

TABLE III

COMPOSITION OF FRACTIONS PRECIPITATED FROM A 30% UREA DISPERSION OF GLUTEN AT DIFFERENT SALT CONCENTRATIONS

Fraction No.	Concentration of $MgSO_4$ expressed in terms of saturation	Weight of protein obtained, approx., gm.	Nitrogen content, %	Humin N %	Amide N %	Arginine N %	Basic N %	Arginine N as % of non-amide N, %	Approximate proportion of each protein in fractions	
									Gliadin, %	Glutenin, %
1	0.13	0.2	15.9							
2	0.16	0.8	16.8	1.0	28.9	8.2		11.4	18	82
3	0.18	4.0	17.3	0.2	25.7	8.0	13.9	10.8	32	68
4	0.21	5.5	17.3	0.1	23.8	7.6	13.0	10.0	50	50
5	0.27	5.5	17.4	0.1	26.1	6.7	11.1	9.1	70	30
6	Approx. 0.5	2.0	16.7	0.2	33.0	5.8		8.7	80	20
Gliadin*					25.8	5.8		7.8		
Glutenin**					19.5	9.8		12.2		

**Average from Table I.*

***Average values for urea preparations of glutenin given by Cook and Alsberg (5).*

It is evident, from the weights of the different fractions obtained, that not more than a third of the original gluten was recovered. The reported values are by no means quantitative, however, since some loss of each fraction occurred in removing the protein from the centrifuge bowl, and in subsequent manipulations. Further, the yield reported for Fraction 6 represents only a part of the protein remaining in solution after the precipitation of Fraction 5. The magnitude and irregularity of the amide nitrogen values indicate that some urea remained sorbed on the protein, as was to be expected. The total nitrogen content reported is therefore too high. Fraction 1, however, appears to have contained some non-nitrogenous impurities. The arginine and basic nitrogen contents of these fractions, however, show a steady decrease as the salt concentration required to precipitate the fraction increases. As gliadin and glutenin contain practically the same amount of the other forms of nitrogen, no progressive change could be expected.

The progressive decrease in the arginine content of the fractions can be attributed to their containing different proportions of gliadin and glutenin. As the presence of urea in the preparations causes an error in the arginine values when expressed as a percentage of the total nitrogen, they were recalculated as a percentage of the non-amide nitrogen. These, and similar values calculated from the arginine nitrogen content of pure gliadin and glutenin, were used to estimate the amount of the two gluten proteins in each of the fractions. The computed values appear in the last two columns of Table III. It is evident from these figures that the precipitate obtained at the lowest salt concentration contains the highest percentage of glutenin, and, in succeeding precipitates, the amount of gliadin increases almost linearly with the salt concentration used for precipitation up to 0.27 of saturation. The precipitate obtained at half saturation still contained glutenin according to the analysis.

It is likely that the protein which remained in solution above this salt concentration was largely gliadin.

In the earlier paper (5) it was concluded that three precipitations were sufficient for removing the gliadin. The data given in Table III bear out this conclusion. The precipitate obtained at 0.16 of saturation contained about 18% of gliadin. This precipitate was not washed with water. Assuming the same degree of separation in two subsequent precipitations, this would reduce the gliadin content of the final precipitate to about 2%. Furthermore, the washing incidental to each precipitation would remove still more of the gliadin.

The occurrence of glutenin in the precipitates obtained at high salt concentrations can be explained by assuming: (a) that this protein is similar to gliadin in that it consists of a number of fractions of different solubilities; or (b) that there is an intimate relationship between the two proteins, the more insoluble glutenin being protected from precipitation by the gliadin. The effect of heat treatment on a whole gluten dispersion was consequently undertaken before attempting similar studies on its component parts.

The Heat Denaturation of Gluten Dispersions

A gluten dispersion was prepared from 170 gm. of wet gluten, 30 gm. of solid urea, and 700 cc. of neutral 30% urea solution. This was allowed to stand for 36 hr. at 0° C. to permit complete dispersion before passing it through a Sharples supercentrifuge to remove the starch. As urea solutions tend to become alkaline on being heated, it was necessary to add a suitable buffer to the solution. Potassium dihydrogen phosphate—disodium hydrogen phosphate ($KH_2PO_4 - Na_2HPO_4 . 12H_2O$) mixtures were chosen for this purpose. As different samples of urea vary somewhat in their reaction, the two components of the buffer system were made up separately in double concentration in 30% urea solution, and these mixed to give a pH of 6.85, as determined experimentally with a quinhydrone electrode. Equal volumes of the protein dispersion and the combined buffer were then mixed. This procedure diluted both the buffer solution and the protein dispersion to one-half their original concentration.

It was not necessary that the exact protein concentration of the dispersion be known, since the effect of any particular treatment could be determined only from the relative values observed. However, attempts were made to determine the amount of protein in dispersion by comparing its solid content with that of the dispersion medium. The results indicated a protein concentration of about 3%, but it was found difficult to check these determinations accurately owing to the large solid content of the solution as compared with the amount of protein it contained.

The effect of storage and hydrogen ion concentration on the viscosity of the above gluten dispersion was studied before investigating the effect of heat treatment.

In the first experiment the hydrogen ion concentration after mixing the protein and buffer solutions was equal to 6.95 pH. Viscosity determinations

were made on this dispersion immediately and at various intervals during storage at 5° C. It should be noted that about 40 hr. had elapsed from the time the gluten was first placed in the urea solution until this experiment was started. The results, which are presented in Table IV, confirm the earlier observations (5) that neither the hydrogen ion concentration nor the viscosity of the dispersion change on being stored, for periods up to 101 hr. Apparently the urea solution causes no further change in the protein after complete dispersion has taken place.

TABLE IV

EFFECT OF TIME OF STORAGE ON THE VISCOSITY OF AN APPROXIMATELY 3% GLUTEN DISPERSION IN 30% UREA-BUFFER SOLUTION*

Time of storage at 5° C., hr.	pH	Viscosity, centipoises	Time of storage at 5° C., hr.	pH	Viscosity, centipoises
Initial	6.95	3.38	27.5	7.00	3.28
4.0	—	3.34	51.5	6.95	3.32
10.0	6.95	3.29	101.0	6.95	3.34

**The gluten had been placed in the urea solution about 40 hr. before this experiment was started, to permit complete dispersion of the protein.*

The effect of hydrogen ion concentration was studied only in the region of neutrality. The composition, pH and viscosity of the protein dispersions used are given in Table V. The results indicate a slight decrease in viscosity as the reaction becomes more alkaline, but there is no evidence of an isoelectric point within the range studied. Since the dispersion medium had a different composition for each hydrogen ion concentration, viscosity determinations were also made on identical solutions without dispersed protein. These values showed practically no change, varying only from 1.19 centipoises at pH 6.00 to 1.20 centipoises at pH 7.35.

TABLE V

EFFECT OF HYDROGEN ION CONCENTRATION ON THE VISCOSITY OF AN APPROXIMATELY 3% GLUTEN DISPERSION IN DIFFERENT 30% UREA-BUFFER SOLUTIONS

Composition of dispersion			pH	Viscosity of final dispersion 1+2+3, centipoises
Solution 1 (9.078 gm. KH_2PO_4 in 500 cc. 30% urea), cc.	Solution 2 (11.876 gm. $Na_2HPO_4 . 12H_2O$ in 500 cc. 30% urea), cc.	Solution 3 (Approximately 6% dispersion of gluten in 30% urea),* cc.		
48.75	1.25	50.00	6.00	3.44
47.50	2.50	50.00	6.00	3.44
40.00	10.00	50.00	6.35	3.45
30.00	20.00	50.00	6.65	3.42
20.00	30.00	50.00	6.95	3.34
10.00	40.00	50.00	7.35	3.28

**The protein concentration in the final dispersion 1+2+3 would, therefore, be approximately 3%.*

The influence of heating on the viscosity of this gluten dispersion was determined by heating it in long narrow test tubes, which were stoppered tightly, and placed in a water bath at 70±0.5° C. for varying periods of time. The results obtained are presented in Table VI. It is evident that the viscosity falls off very rapidly during the first hour of heating. After that, a further, but much slower, decrease in viscosity results. Judging from the last experiment on the effect of hydrogen ion concentration, it seems probable that the decrease in viscosity after the first hour is due to the dispersion becoming more alkaline. To give a better picture of these results, the viscosity of the dispersion has been plotted against the time of heating and the resulting chart is shown in Fig. 1.

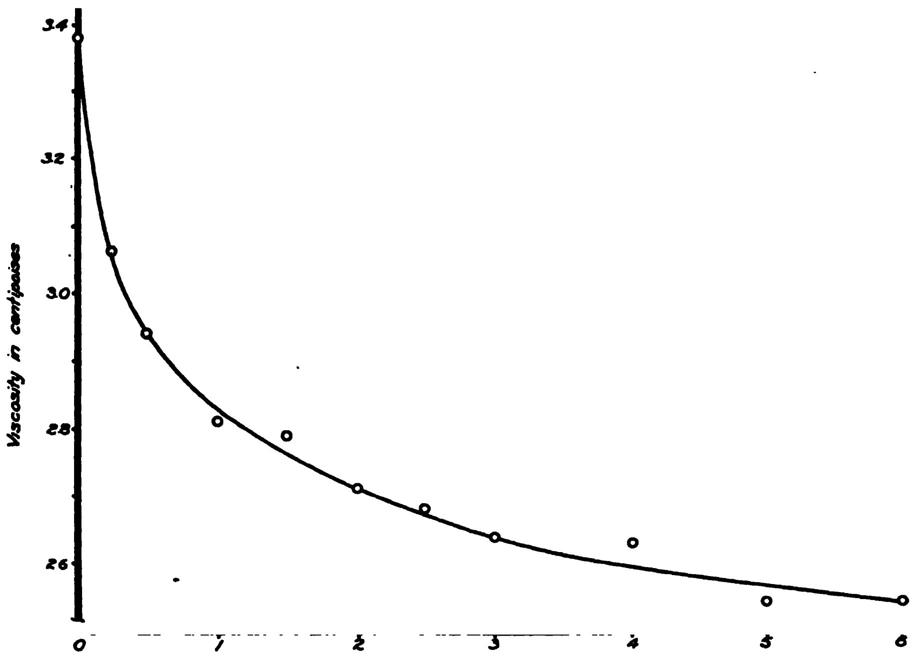


FIG. 1. *The influence of the time of heating at 70°C. on the viscosity of an approximately 3% dispersion of gluten in buffered 30% urea solution.*

TABLE VI

EFFECT OF HEAT TREATMENT ON THE VISCOSITY OF AN APPROXIMATELY 3% GLUTEN DISPERSION IN 30% UREA-BUFFER SOLUTION

Time of heat treatment at 70° C.		pH	Viscosity, centipoises	Time of heat treatment at 70° C.		pH	Viscosity, centipoises
hr.	min.			hr.	min.		
Not heated		6.95	3.38	2	30	7.20	2.68
0	15	7.00	3.06	3	00	7.35	2.64
0	30	6.95	2.94	4	00	7.45	2.63
1	00	7.00	2.81	5	00	7.65	2.54
1	30	7.05	2.79	6	00	7.75	2.54
2	00	7.10	2.71				

Heat Denaturation of Gliadin

Having established a definite decrease in the viscosity of gluten dispersions heated at 70° C., the investigation was continued to determine the effect of various heat treatments on the properties of purified gliadin. This protein was heated in two forms: (a) solid, of natural moisture content, since this approximates the conditions under which the baking quality of flour is altered; and (b) dispersed in urea-buffer solutions, to provide data comparable with those obtained for gluten dispersions. The gliadin preparations described earlier provided experimental material. The heat treatment of an alcoholic dispersion of gliadin was not undertaken, since the results of Gottenberg and Alsberg (10) indicate that gliadin dispersed in 60% alcohol was not significantly altered by being held at 58° C.

The plan of experiment followed with the solid material was to heat, dry, and finally disperse the gliadin in 60% alcohol. The C.P.T. and viscosity of the resultant dispersion were taken as criteria of change. Preliminary experiments had shown that the protein could not be completely dispersed if it had been subjected to temperatures over 80° C. Samples of high moisture content were rendered insoluble at even lower temperatures. Subsequent experiments were therefore confined to samples of natural moisture content (12 to 14%) at a temperature of 70° C.

The procedure followed was to weigh accurately into weighing bottles samples of gliadin somewhat in excess of one-half gram. The bottles were then sealed with a hard cement, and the samples heated in a water bath for various periods at $70 \pm 0.5°$ C. On removal from the bath, the bottles were again weighed to determine if any loss in moisture had taken place, opened, and the gliadin samples dried over phosphorus pentoxide *in vacuo* at room temperature. When the samples had reached constant weight, the weight of protein in the bottles was adjusted accurately to 0.5000 gm. and 5 cc. of 60% alcohol added to each bottle. The stoppers were then replaced and sealed, and the gliadin allowed to disperse for three days at room temperature with occasional shaking. It was observed that the samples exposed to the longest heat treatments dispersed more slowly than the others. When all the samples were completely dispersed, the volume was adjusted to exactly 10 cc. by the addition of 60% alcohol, allowed to stand for another day, and samples taken for viscosity and C.P.T. measurements. The results obtained are given in Table VII, each value reported being the average of at least duplicate determinations.

It is evident from the moisture data that the samples dried out somewhat while being heated. The C.P.T., however, shows no significant change for either preparation, although the regular and progressive increase in the temperatures for Preparation 1 suggests that these samples may have become slightly less soluble. The viscosity determinations are somewhat irregular, a result which must be attributed to error as no progressive change is evident. The conclusion to be drawn from this experiment is that gliadin is not significantly altered in C.P.T. or viscosity by being heated at 70° C. for several hours at its natural moisture content.

TABLE VII

EFFECT OF HEAT-TREATING GLIADIN OF NORMAL MOISTURE CONTENT ON ITS SUBSEQUENT C.P.T. AND VISCOSITY IN 60% ALCOHOL

Time of treatment at 70° C. hr. min.	Moisture content Before heating %	After heating %	Average %	C.P.T. 5% solution, °C.	Viscosity 5% solution, centipoises
Preparation No. 1					
Not heated	14.3	14.0	14.2	8.6	5.11
Not heated	14.0	13.8	13.9	8.4	5.47
0 15	14.1	13.9	14.0	8.8	5.22
0 30	14.2	14.0	14.1	9.0	—
1 00	14.0	13.8	13.9	9.1	5.31
1 30	14.0	13.5	13.8	9.2	—
2 00	14.0	13.4	13.7	9.4	5.30
2 30	14.1	13.4	13.8	9.6	5.46
4 00	14.0	12.8	13.4	9.8	5.30
5 00	13.9	12.4	13.2	9.6	5.57
6 00	14.0	12.4	13.2	9.6	—
Preparation No. 2					
Not heated	13.2	12.7	13.0	10.7	5.95
Not heated	12.5	12.3	12.4	10.9	5.86
0 15	12.8	12.6	12.6	11.2	6.05
0 30	12.9	12.5	12.7	11.1	6.21
1 00	14.7*	11.4	13.0*	11.0	6.03
1 30	13.4	12.4	12.9	11.1	6.03
2 00	13.3	10.2	11.8	11.3	6.06
2 30	12.8	11.7	12.2	11.0	6.17
3 00	14.3	11.3	12.8	11.0	5.97
4 00	12.4	11.9	12.2	11.2	6.01
5 00	12.2	11.2	11.7	11.3	6.27
6 00	13.7	9.4	11.6	11.0	6.10

**Doubtful values.*

In order to obtain information comparable with that obtained for whole gluten, 4% dispersions of gliadin were prepared in buffered 30% urea solutions. The two components of the buffer system were made up separately, and mixed to give a pH of 6.85. The protein dissolved within two hours, but the dispersions were allowed to stand for 36 hr. at 0° C. before being heated. They were then transferred to long, narrow test tubes, which were stoppered tightly and put in a water bath at $70 \pm 0.5°$ C. for the required length of time. When removed from the bath, the tubes were cooled quickly and the pH and viscosity of the protein dispersions determined immediately. The average values for duplicate determinations are given in Table VIII.

The results show that the dispersion became more alkaline as the time of heat treatment increased, but this change was usually small during the first 1½ to 2 hours. The dispersion of Preparation 3 was originally much more acidic than the others, and as the buffer solution originally had a

TABLE VIII

EFFECT OF HEAT TREATMENT ON THE VISCOSITY OF 4% GLIADIN DISPERSIONS IN 30% UREA SOLUTIONS

Time of treatment at 70° C. hr.	min.	pH	Viscosity, centipoises
Preparation No. 1			
Not heated		6.90	2.59
0	30	6.95	2.52
1	30	7.10	2.54
2	30	7.35	2.58
Preparation No. 2			
Not heated		6.95	3.05
0	15	6.90	2.97
0	30	6.95	2.88
1	00	7.00	2.92
2	00	7.15	2.87
3	00	7.35	2.79
4	25	7.90	2.84
Preparation No. 3			
Not heated		6.45	2.64
0	15	6.45	2.63
0	30	6.50	2.60
1	00	6.70	2.66
1	30	6.85	2.58
2	00	6.95	2.58
2	30	7.10	2.58
3	00	7.15	2.61
4	00	7.35	2.58
5	00	7.55	2.56
6	00	7.80	2.61
Preparation No. 4			
Not heated		6.85	5.91
1	30	7.05	4.71
Preparation No. 5			
Not heated		6.90	3.24
1	30	7.05	3.07
Dispersion medium without protein			
Not heated		—	1.18
6	00	—	1.22

pH of 6.85, this change must be attributed to the protein. The only apparent reason for this acidic property is that some acetic acid remained sorbed on the gliadin, from its initial extraction with the solvent.

The viscosity of Preparations 1 and 3 was apparently unaltered by heat. Dispersions of the remaining preparations, however, showed a decrease in viscosity after being heated. Only one period of heat treatment was possible with Preparations 4 and 5 owing to lack of material. The results given at the foot of the table show the initial viscosity of the dispersion medium and its viscosity after six hours' heating at 70° C. This treatment increased the viscosity somewhat, but not sufficiently to affect the results obtained with the protein dispersions.

The decrease in viscosity of the gluten and gliadin dispersions, after 1½ hours' heating, has been expressed as a percentage in Table IX. It should be noted that the gluten dispersion and the gliadin dispersions are perhaps not strictly comparable since their concentrations are different. Nevertheless the heat treatment decreased the viscosity of the less concentrated gluten dispersion more than it did that of the gliadin dispersions with the exception of Preparation 4 which had a high initial viscosity. This indirect evidence indicates that glutenin is the component of gluten most easily altered by heat. More direct information as to the effect of heat on glutenin awaits further investigation.

The different physical properties of these gliadin preparations initially (see Table II) have been harmonized with the work of Haugaard and Johnson (12) who have shown that gliadin can be fractionated. Evidently the same considera-

TABLE IX

DECREASE IN VISCOSITY OF GLUTEN AND GLIADIN DISPERSIONS AFTER HEATING AT 70° C.

Material	Viscosity		Decrease	
	Initial, centipoises	After 1½ hours' heating, centipoises	Centipoises	%
Gluten	3.38	2.79	0.59	17.4
Gliadin				
Preparation 1	2.59	2.54	0.05	1.9
Preparation 2	3.05	2.87*	0.18	5.9
Preparation 3	2.64	2.58	0.06	2.3
Preparation 4	5.91	4.71	1.20	20.3
Preparation 5	3.24	3.07	0.17	5.2

**This determination was made after two hours' heating.*

tions will explain the apparent anomalies as to the effect of heat treatment. Thus Preparations 1 and 3, having the lowest viscosities and C.P.T.'s originally, show no significant change in viscosity on being heated. The viscosities of Preparations 2, 4 and 5, however, were decreased by heat treatment, the extent of the change increasing with the initial viscosity and the C.P.T. of the sample. The more insoluble fractions of gliadin are therefore the first to be affected by heat, the magnitude of the change caused by a given heat treatment depending on the solubility of the fraction.

A further study of the heat denaturation of gliadin was made by investigating the properties of alcoholic extracts from heated flours. Samples of two flours milled from hard red spring wheat were placed in a saturated atmosphere at 0° C. until they had a moisture content of 17.9 and 19.1% respectively. Samples of approximately six grams each were then packed tightly into glass tubes of about 0.5 cm. internal diameter, the tubes sealed, and then heated for varying periods of time at 96.2° C. The small tubes were used to permit rapid and thorough heating of the whole sample. After being heated, the flour samples were transferred to tared dishes and dried at 98° C. *in vacuo.* (It will be shown later that the heat treatment incidental to drying had no effect on the properties under investigation.) Five-gram samples of the dry flour were then weighed into test tubes, 25 cc. of 60% alcohol (by volume) added, and the tubes stoppered. They were allowed to stand for 32 hr. at room temperature, and then shaken for six hours at 45° C., cooled, and the supernatant liquid removed after centrifuging. The nitrogen content and C.P.T. of the extract were then determined. The results are presented in Table X, and represent the average of duplicate heat treatments on each flour.

The results obtained were essentially the same for both flours. The irregularities in moisture content can probably be attributed to evaporation losses in transferring the flour from the tubes to the drying dishes, since the actual heat treatment was performed in sealed tubes. Comparison of the first two values for each flour shows that drying at 98° C. *in vacuo* had no effect on the

TABLE X

EFFECT OF HEAT-TREATING FLOUR ON THE AMOUNT OF GLIADIN EXTRACTED BY 60% ALCOHOL AND THE C.P.T. OF THE EXTRACT

Time of heat treatment at 96.2° C. hr.	min.	Moisture content of flour %	Protein concentration in 60% alcohol extract %	C.P.T. of extract °C.
Flour I				
Dried over $CaCl_2$ *in vacuo.* No heat treatment given		17.9	2.9	12.0
Dried at 98° C. *in vacuo* immediately		18.4	3.0	12.0
0	15	17.6	1.3	6.5
0	30	17.9	0.9	6.0
1	00	17.8	1.0	7.0
2	00	17.7	0.6	6.0
4	00	17.6	0.4	6.0
8	00	17.3	0.5	6.5
Flour II				
Dried over $CaCl_2$ *in vacuo.* No heat treatment given		19.1	2.4	11.0
Dried at 98° C. *in vacuo* immediately		19.4	2.4	11.0
0	15	18.7	0.9	6.5
0	30	19.1	0.6	6.5
1	00	18.8	0.7	6.5
2	00	18.4	0.5	6.5
4	00	18.2	0.4	6.0
8	00	18.3	0.4	6.5

properties studied. The heated samples were consequently dried at this temperature. The figures for the amount of protein extracted by the alcohol were calculated from the nitrogen content of the extract. These values are also somewhat irregular, but in general they show a progressive decrease with increased time of heating. The C.P.T. of the extracts is distinctly lower for all the heated flours. There is however no progressive change in this property with increasing time of treatment. Dill and Alsberg (6) have shown that the C.P.T. is largely independent of the gliadin concentration, but, as the concentration varied considerably in these extracts, this point was tested by adding an equal volume of 60% alcohol to those extracts which had a high C.P.T. and the determination repeated. No change in C.P.T. resulted,

Comparison with the controls shows that 15 min. heat treatment lowered the gliadin content of the extract about one-half, and decreased the C.P.T. about 5° C. This bears out the earlier conclusion that the gliadin fractions of low solubility are the first to be altered by heat. Heating the flour for longer periods caused a progressive, but less marked, decrease in the amount of protein

extracted by alcohol, but made no significant difference in the C.P.T. of the extract. The lower protein content of the extracts from the severely heated samples must therefore be attributed to incomplete extraction, probably due to occlusion by the increasingly coagulated glutenin and less soluble gliadin fractions.

Conclusions

Gliadin samples prepared by several different methods were essentially the same chemically. The solubility and viscosity of these samples, however, showed considerable variation. There are two possible explanations of this: (a) that gliadin consists of a number of fractions; or (b) that it was partly denatured in preparation and its physical properties altered. Since Haugaard and Johnson (12) have shown that gliadin can be fractionated, the first explanation is accepted. The possibility of some denaturation having occurred cannot be excluded, however, as the gliadin samples all gave a positive nitroprusside test, which may indicate denaturation if gliadin does not give this test originally. That it does seems unlikely (5).

The fractionation of gluten by precipitation from urea solutions at different salt concentrations, shows that under these conditions there is no clear cut distinction between the solubilities of glutenin and gliadin. From a chemical standpoint these fractions decreased in their arginine content with increasing salt concentration at which they were precipitated. This can be explained by the protection of the glutenin by the gliadin, the former being precipitated only when the salt concentration is sufficient to precipitate the more insoluble fractions of the latter. On the other hand, glutenin as well as gliadin may exist as a number of fractions, similar chemically but different physically.

Gliadin of natural moisture content is not altered appreciably in its C.P.T. or viscosity by being heated at 70° C., although more severe heating renders it insoluble. From the viscosity changes which occur when dispersions of whole gluten and gliadin are heated at 70° C. in urea-buffer solutions, it is concluded that glutenin is the first protein to be affected by heat, next the gliadin fractions of low solubility, and finally under severe conditions all of the gliadin may be denatured. This conclusion is supported in part by the fact that 60% alcohol extracts from heated flours contained less gliadin, but these extracts had lower C.P.T.'s than the controls, showing that the extracted gliadin had a greater solubility than that extracted from unheated flour. More direct evidence as to the effect of heat on glutenin awaits further investigation.

Acknowledgments

The author is indebted to Dr. C. L. Alsberg, Director of the Food Research Institute, Stanford University, California, for his counsel throughout the execution of this investigation; and to Dr. R. Newton, Professor of Field Crops, University of Alberta, for his ready assistance and advice.

References

1. Alsberg, C. L. and Griffing, E. P. The heat coagulation of gluten. Cereal Chem. 4: 411-423. 1927.

2. BLISH, M. J. and SANDSTEDT, R. M. An improved method for the preparation of wheat gliadin. Cereal Chem. 3: 144-149. 1926.
3. BLISH, M. J. and SANDSTEDT, R. M. The nature and identity of wheat glutenin. J. Biol. Chem. 85: 195-206. 1929.
4. CHICK, H. and MARTIN, C. J. The 'heat coagulation' of proteins. J. Physiol. 40: 404-430. 1910.
5. COOK, W. H. and ALSBERG, C. L. The preparation of glutenin in urea solutions. Can. J. Research, 5: 355-374. 1931.
6. DILL, D. B. and ALSBERG, C. L. Preparation, solubility and specific rotation of wheat gliadin. J. Biol. Chem. 65: 279-304. 1925.
7. GEDDES, W. F. Chemical and physico-chemical changes induced in wheat and wheat products by elevated temperatures—I. Can. J. Research, 1: 528-558. 1929.
8. GEDDES, W. F. Chemical and physico-chemical changes induced in wheat and wheat products by elevated temperatures—II. Can. J. Research, 2: 65-90. 1930.
9. GORTNER, R. A., HOFFMAN, W. F. and SINCLAIR, W. B. Physico-chemical studies on proteins. III. Proteins and the lyotropic series. Coll. Sym. Mono. 5: 179-198. 1928.
10. GOTTENBERG, M. J. and ALSBERG, C. L. The behavior of the prolamines in mixed solvents. III. The denaturation of wheat gliadin. J. Biol. Chem. 73: 581-586. 1927.
11. HARRIS, L. J. On the existence of an unidentified sulphur grouping in the protein molecule. Part I. On the denaturation of proteins. Proc. Roy. Soc. B94: 426-441. 1923.
12. HAUGAARD, G. and JOHNSON, A. H. The fractionation of gliadin. Compt. rend. trav. lab. Carlsberg. 18 (2): 1-138. 1930.
13. HERD, C. W. A study of some methods of examining flour, with special reference to the effects of heat. I. Effects of heat on flour proteins. Cereal Chem. 8: 1-23. 1931.
14. HOPKINS, F. G. Denaturation of proteins by urea and related substances. Nature, 126: 328-330 and 383-384. 1930.
15. INTERNATIONAL CRITICAL TABLES. vol. 5, 1929.
16. KENT-JONES, D. W. A study of the effect of heat upon wheat and flour, especially in relation to strength. Thesis presented at London University. 1926.
17. NATIONAL RESEARCH COUNCIL OF CANADA. The drying of wheat. Report 24. 1929.
18. OSBORNE, T. B. The proteins of the wheat kernel. Carnegie Inst. Wash. Pub. 84. 1907.

OCCURRENCE AND SPORULATION OF *HELMINTHOSPORIUM SATIVUM* P.K.B. IN THE SOIL[1]

By A. W. Henry[2]

Abstract

Helminthosporium sativum, one of the most prevalent fungous pathogenes affecting the roots and other parts of wheat and related grass plants, grows and fruits readily as a saprophyte on various substrata. It might therefore be expected that it would be found in abundance in the soil. Attempts to isolate it directly from field soils by plating have been successful, but only in a small percentage of the trials. Failures are apparently largely attributable to the fact that this fungus does not sporulate commonly in ordinary field soils. This has been shown by direct microscopic examination of the soil and by artificial cultures. From a study of the latter it has been found that *H. sativum* will sporulate quite readily on several different soil types including sand if they are sterilized, but not if they are not sterilized.

The fact that soils capable of supporting sporulation of this fungus may be rendered ineffective by adding small amounts of unsterilized soil, suggests that sporulation is inhibited by the saprophytic micro-organisms of the soil. It would appear that if *H. sativum* occurs in the soil, it must be present chiefly in the form of mycelium. Inability to form spores probably lessens the capability of this fungus to survive as well as to multiply in the soil.

Introduction

Although it is commonly assumed that root-rotting pathogenes of wheat and other cereals live and multiply in the soil, actually little is known regarding their saprophytic life in the soil. As such knowledge appears fundamental to an intelligent attack on root-rot problems, investigations for the purpose of accumulating further information of this sort were planned. The object of the experiments reported in this paper was to determine if *H. sativum*, one of the most prevalent and destructive of these fungous pathogenes, exists in soils which have been cropped to wheat and if so in what form.

It would seem from an examination of the literature that species of *Helminthosporium* are not particularly common in the soil. No references reporting the direct isolation of *H. sativum* from the soil have come to the writer's attention. It should be noted, however, that this species was not described until 1910 (9) and that Beckwith (1) in 1911 reported the isolation of a *Helminthosporium* from soil which had been constantly cropped to wheat for forty years. Bolley (2, 3) moreover considered *Helminthosporium* one of the genera responsible for wheat-sick soils in North Dakota. It is quite probable that *H. sativum* was the principal species encountered by these workers. In fact Drechsler (6) includes the *Helminthosporium* sp. of Beckwith and Bolley under the synonyms of *H. sativum.*

The writer (8) in 1921-22 attempted to isolate *H. sativum* directly from the soil by plating methods at St. Paul, Minnesota, but was unsuccessful. In a summary of soil fungi published in 1927 by Gilman and Abbott (7) *H. sativum*

[1] *Manuscript received July 24, 1931.*
Contribution from Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada.

[2] *Associate Professor of Plant Pathology, University of Alberta.*

is not included among the species of *Helminthosporium* which had been reported up to that time as occurring in the soil. They, however, report the occurrence of three other species of *Helminthosporium* in the soil, namely *H. subulatum*, *H. interseminatum* and *H. anomalum*.

Occurrence of *H. sativum* in Alberta Soils

In order to ascertain if *H. sativum* occurs in Alberta soils, samples of the latter were first obtained from wheat fields in different parts of the province. Most of these were collected during August 1928 and were taken from fields in which the plants were still standing or in which the stubble remained. The samples were taken from between the drill rows and at the same time collections were made of adjacent wheat-culm bases. Attempts were then made in the laboratory to isolate *H. sativum* from both soil and stubble in order to determine if any relationship exists between the prevalence of this fungus on wheat and its occurrence in the soil.

In taking the soil samples, precautions were observed to avoid the introduction of the fungus from outside sources such as the air or neighboring plants. The surface soil to a depth of about an inch was removed with a sterile steel spatula. The spatula was then sterilized again by dipping in alcohol and flaming, and used for taking the soil sample and introducing it into a sterile test tube. In the laboratory the samples were prepared for plating as follows: A small thimbleful of each was worked through a wire screen ($\frac{1}{16}$ in. mesh) attached to the bottom of a one-holed cork inserted in a 200-cc. Erlenmeyer flask containing 20 cc. of sterile tap water. This apparatus had previously been sterilized at 15 lb. pressure in an autoclave. After the introduction of the soil, the flask was shaken for five minutes. Dilutions of 1-2000 and in some cases of 1-4000 were then made in other flasks containing sterilized tap water and platings from these were made after adding 1-cc. portions to tubes of acid agar as suggested by Waksman (11). Pieces of stubble or wheat bases were surface sterilized by dipping them into 70% alcohol, then placing them in a 1-1000 mercuric chloride solution for 2-3 min., and dipping them again into 70% alcohol. They were then transferred with sterile forceps to acid agar.

It will be noted from Table I, in which the results are summarized, that *H. sativum* was not obtained directly from the soil of any of the wheat fields from different parts of the province. It was, however, isolated from adjacent wheat-culm bases in about 9% of the trials.

Another series of soil samples was taken from the black soil of the Edmonton district. It was thought that this soil, being high in organic matter, might be more suitable for the growth of *H. sativum* than some of the others examined.

These samples were collected monthly over a period of more than a year from land in wheat or bearing wheat stubble and from fallow land. The results are given in Tables II and III. From Table II it is apparent that while *H. sativum* was isolated from two of the soil samples it was not obtained as often as might have been expected from its prevalence on adjacent wheat-culm bases. It was obtained for instance from only two of the soil samples as compared with nine of the adjacent stubble samples. There is of course the possibility that

TABLE I

RESULTS OF ATTEMPTS TO ISOLATE *H. sativum* FROM THE SOIL AND ADJACENT WHEAT STUBBLE COLLECTED IN DIFFERENT PARTS OF ALBERTA

No. of sample	Place collected	Date collected	Isolations of *H. sativum*			
			Soil		Stubble	
			No. of colonies	No. of plates	No. of colonies	No. of pieces stubble
45	Lacombe	Aug. 4, 1928	0	2	0	5
65	Big Valley	Aug. 6, 1928	0	3	0	6
83	De Winton	Aug. 7, 1928	0	3	1	6
91	Cayley	Aug. 8, 1928	0	2	1	6
94	Claresholm	Aug. 9, 1928	0	3	1	6
103	Kipp	Aug. 9, 1928	0	3	1	6
149	Stanmore	Aug. 16, 1928	0	3	2	6
155	Scotfield	Aug. 16, 1928	0	3	1	6
165	Wainwright	Aug. 17, 1928	0	3	1	6
176	Vegreville	Aug. 18, 1928	0	2	0	6
184	Breton	Aug. 23, 1928	0	2	0	6
190	Tofield	Aug. 23, 1928	0	4	0	6
195	Ryley	Aug. 23, 1928	0	3	0	6
199	Holden	Aug. 24, 1928	0	4	0	6
217	Ohaton	Aug. 24, 1928	0	4	0	3
232	Spruce Grove	Aug. 31, 1928	0	3	0	8
246	Spruce Grove	Nov. 26, 1928	0	7	2	19
Totals			0	54	10	113

TABLE II

RESULTS OF ATTEMPTS TO ISOLATE *H. sativum* FROM THE SOIL AND ADJACENT WHEAT STUBBLE COLLECTED AT EDMONTON

No. of sample	Date collected	Isolations of *H. sativum*			
		Soil		Stubble	
		No. of colonies	No. of plates	No. of colonies	No. of pieces stubble
241	Oct. 5, 1928	2	8	0	6
243	Nov. 7, 1928	0	6	6	10
249	Dec. 20, 1928	0	5	3	10
252	Jan. 29, 1929	0	7	1	3
253	Feb. 28, 1929	0	9	1	4
255	Mar. 30, 1929	1	6	1	3
257	April 30, 1929	0	4	0	6
260	May 31, 1929	0	3	0	9
262	June 30, 1929	0	2	0	9
264	July 31, 1929	0	3	3	5
265	Aug. 31, 1929	0	6	3	9
267	Sept. 30, 1929	0	4	0	9
269	Oct. 31, 1929	0	6	0	12
271	Nov. 30, 1929	0	6	0	12
273	Dec. 31, 1929	0	4	2	10
275	Jan. 31, 1930	0	6	4	15
Totals		3	85	24	132

the isolations from the soil in these instances actually came from spores or other parts of the fungus produced on wheat roots in the soil, even though the

precaution of screening out the roots was taken. In the case of summer-fallow where no wheat plants were present to harbor the organism it might be expected that the fungus would be less abundant. The results given in Table III show that it was isolated once from sixteen samples of summer-fallow

TABLE III *

RESULTS OF ATTEMPTS TO ISOLATE *H. sativum* FROM SUMMER-FALLOW SOIL AT EDMONTON

No. of sample	Date collected	Isolations of *H. sativum*		No. of sample	Date collected	Isolations of *H. sativum*	
		No. of colonies	No. of plates			No. of colonies	No. of plates
244	Nov. 7, 1928	1	6	261	June 30, 1929	0	3
248	Dec. 20, 1928	0	4	263	July 31, 1929	0	2
250	Dec. 20, 1928	0	5	264	Aug. 31, 1929	0	6
251	Jan. 29, 1929	0	7	266	Sept. 30, 1929	0	4
254	Feb. 28, 1929	0	9	268	Oct. 31, 1929	0	6
256	Mar. 30, 1929	0	6	270	Nov. 30, 1929	0	6
257	April 30, 1929	0	4	272	Dec. 31, 1929	0	4
259	May 31, 1929	0	3	274	Jan. 31, 1930	0	6
				Totals		1	81

soil as compared with thrice from a similar number of samples of soil cropped to wheat. The difference is not sufficient, however, to warrant the conclusion that *H. sativum* is less abundant in the summer-fallow soil. Since the plate method of isolation is probably dependent for its success largely on the presence of spores as has been pointed out by Conn (5), it seemed advisable to ascertain to what extent *H. sativum* sporulates in the soil.

Microscopic Examination of Soils for *H. sativum* Spores

As the spores of *H. sativum* are large and readily distinguished from other fungous spores it was decided to make a direct microscopic examination of soil samples to ascertain if these spores occur under natural conditions in field soils. For this purpose soil samples were collected in a similar manner to that previously described except that in this case a sterile cork borer was used instead of a spatula to take the samples. One set of samples was obtained from the four replicates of a set of plots which had borne three crops of spring wheat in succession. Thirteen distributed samples were taken from each plot, making 52 samples in all. Another set of samples was taken from a large nearby block of fallow soil. Forty-two samples of approximately ½ cu. in. each were taken from different parts of this plot. In the laboratory, each sample was then prepared for examination as follows:— A small thimbleful was measured out in the base of a test tube and transferred to a clean sterile test tube. Then 5 cc. of distilled water was added and the contents shaken for one-half minute. After settling for one minute, 0.07 cc. of the suspension was drawn off with one of the pipettes of a haemacytometer set and transferred to a

clean cover slip. This was then inverted over the counting chamber of the set and immediately examined with the microscope. A careful examination of the samples, 94 in all, failed to reveal spores of *H. sativum* in any of them.

Sporulation of Artificial Cultures of *H. sativum* in Alberta Soils

In order to determine if *H. sativum* could produce spores in the soil it was decided to prepare artificial soil cultures and then to examine them by means of the haemocytometer. Several soils were selected for this purpose, namely, black soil typical of the Edmonton district, brown soil from Brooks, grey soil from Cooking Lake and fine sand from Edmonton. Moisture was added to the water-holding capacity of each, in 200-cc. Erlenmeyer flasks each containing 50 gm. of soil. One series of these was sterilized while another was left unsterilized. Then a tiny bit of a non-sporulating colony of *H. sativum* on water agar was introduced into each and placed on the surface of the soil in a marked position near one side of the flask. After the organism had been allowed time to develop, equal portions of the soil were examined for the presence of spores. Samples were removed from the flask by means of a piece of brass tubing soldered to a steel rod. Two samples of equal surface area, approximately ⅛ in. in depth, were thus removed about one inch from the piece of agar and mixed in a test tube with 2 cc. of sterile distilled water. After shaking for one minute, each tube was allowed to stand two minutes. Then 0.07 cc. of the suspension was drawn off with a pipette and examined as before by means of the haemacytometer. The results are shown in Table IV.

FIG. 1. *Spores of the same monosporous culture of H. sativum grown on sterilized black soil (left), and on potato dextrose agar (right.)*

It will be seen that the fungus sporulated freely on all samples of sterilized soil but most abundantly on the black soil, though it was not intended to compare the relative sporulation on the different soils, but merely to demonstrate whether the organism could or could not sporulate in the different soils. It is noteworthy that no spores were found in the cultures on any of the unsterilized soils, also that the culture on sterilized soil which produced the fewest spores was contaminated with bacteria. The spores produced on sterilized soil appeared quite normal and germinated vigorously though they were somewhat lighter in color than those produced on potato dextrose agar, Fig. 1. Sporulation on sterilized soil to which organic matter had been added was observed by Christensen (4). Marked differences in the sporulation of different strains of the fungus were also noted by him. In the experiments here reported water only was added to the soil.

TABLE IV

SPORULATION OF *Helminthosporium sativum* ON ALBERTA SOILS

Soil type	Source	Sterilized soil		Unsterilized soil	
		Culture number	Number of spores per cc.	Culture number	Number of spores per cc.
Black	Edmonton	1	8502	21	0
		2	3001	22	0
		3	2834	23	0
		4	1167	24	0
		5	834	25	0
			Av. 3268		Av. 0
Brown	Brooks	6	333	26	0
		7	333	27	0
		8	1667	28	0
		9	2001	29	0
		10	1334	30	0
			Av. 1134		Av. 0
Grey	Cooking Lake	11	1000	31	0
		12	167*	32	0
		13	2167	33	0
		14	667	34	0
		15	333	35	0
			Av. 867		Av. 0
Fine sand	Edmonton	16	834	36	0
		17	333	37	0
		18	4334	38	0
		19	333	39	0
		20	667	40	0
			Av. 1300		Av. 0

**Contaminated with bacteria.*

Another experiment was made using similar methods except that all of the flasks of soil were first sterilized and then the fungus added. Then small quantities of unsterilized soil were introduced into some of the flasks. The amounts added consisted of a trace and one-gram portions. The trace refers to the small amount which adhered to a moist sterilized platinum needle. The results for the Edmonton black soil are shown in Table V. Similar results

TABLE V

EFFECT ON THE SPORULATION OF *H. sativum* OF SMALL ADDITIONS OF UNSTERILIZED SOIL TO STERILIZED SOIL

Soil type	Source	Treatment	Number of spores per cc.
Black	Edmonton	Sterilized	3834
Black	Edmonton	Sterilized	1167
Black	Edmonton	Sterilized + trace unsterilized	0
Black	Edmonton	Sterilized + trace unsterilized	0
Black	Edmonton	Sterilized + 1 gm. unsterilized	0
Black	Edmonton	Sterilized + 1 gm. unsterilized	0

were obtained for the other types referred to in Table IV. As will be noted in Table V sporulation occurred in the sterilized soil alone, but not in the sterilized sample which received either a trace or one-gram portions of unsterilized soil. Evidently the saprophytic micro-organisms of the soil introduced with the unsterilized soil prevented the sporulation of *H. sativum.*

Discussion

While the results obtained indicate that *H. sativum* may occur in field soils which have been cropped to wheat one or more years they do not indicate that it is abundantly present in such soils. It is possible, however, that this fungus may be more common in the soil than the results suggest, if it exists there chiefly in the form of mycelium. As it has been shown that spores of *H. sativum* are very rare if not entirely absent from field soils, it would appear that this fungus if it lives at all in the soil must be largely present in the form of mycelium. The fact that sporulation occurs on sterilized soil but not on unsterilized soil as has been demonstrated, may possibly be explained on the basis of chemical changes in the soil as a result of steam-sterilization, but it would appear that the inhibition of sporulation on unsterilized soil is at least partly attributable to the suppressive action of the normal saprophytic micro-organisms of the soil.

The results may also have a bearing on the survival of the fungus in the soil. If spores are absent, and mycelium only is present, the organism might naturally be expected to be less capable of persisting through periods of adverse conditions, than where both spores and mycelium are present as on most substrata.

References

1. Beckwith, T. D. Phytopathology, 1: 169-176. 1911.
2. Bolley, H. L. Science, 32: 529-541. 1910.
3. Bolley, H. L. N. Dak. Agr. Exp. Sta. Bul. 107: 1-94. 1913.
4. Christensen, J. J. Univ. Minn. Tech. Bul. 37: 1-101. 1926.
5. Conn, H. J. Soil Science, 14: 149-151. 1922.
6. Drechsler, C. J. Agr. Research, 24: 641-739. 1923.
7. Gilman, J. C. and Abbott, E. N. Iowa State Coll. J. Science, 1: 225-345. 1927.
8. Henry, A. W. Univ. Minn. Tech. Bul. 22: 1-71. 1924.
9. Henry, A. W. Can. J. Research, 4:69-77. 1931.
10. Pammel, L. H., King, C. M. and Bakke, A. L. Iowa Agr. Exp. Sta. Bul. 116: 178-190. 1910.
11. Waksman, S. A. Principles of soil microbiology. Williams & Wilkins. Baltimore. 1927.

STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES.

XXXVII. THE FORMATION OF DEXTRAN BY *LEUCONOSTOC MESENTERIOIDES*[1]

By H. L. A. Tarr[2] and Harold Hibbert[3]

Abstract

Four strains of *Leuconostoc mesenterioides* have been studied, and a medium suitable for the production of relatively large amounts of polysaccharide from sucrose has been carefully evolved. All of these organisms brought about polysaccharide formation in nutrient solutions containing sucrose, while two of them showed a slight activity in this respect toward glucose. Melezitose, raffinose, fructose, galactose, lactose, maltose, xylose and glycerol, when employed in the place of sucrose, did not yield any polysaccharide. It is not yet certain if a definite relation exists between the structure of the polysaccharide formed and the sugar utilized, as was found to be the case with levan (8, 9, 10).

Introduction

Pasteur (16) first showed that the slimy fermentation of carbohydrates was due to bacterial action, and assigned to the resulting gummy material the formula $C_{12}H_{20}O_{10}$. Scheibler (12, pp. 461-468) identified a mucilaginous material isolated by him from the juice of sugar beets as being an anhydride of glucose, closely related to starch and dextrin, and therefore named it "dextran." He erroneously believed that the "dextran" slime was formed from the cells of the sugar beet by the action of some enzyme. Jubert (12, p. 462) demonstrated that the slime could be propagated only in beet juice, and that this power of reproduction could be destroyed by heat, or by the addition of phenol, thus giving indirect evidence in support of the idea that the slime formation was caused by a living organism. Mendes (12, p. 462) observed that within the gelatinous masses there were small cells which were able to multiply by fission, thus contributing additional and more conclusive evidence in support of the assumption that the mucilaginous fermentation resulted from the activity of micro-organisms. Durin (5) wholly misinterpreted the phenomenon and decided that the mucilaginous material which he isolated from a vat of German molasses was cellulose, formed from sucrose by the action of the enzymes of the sugar beet. Cienkowski (12, p. 463) recognized the slime produced by this type of fermentation as a true zoogleal formation, and classified the causal organism as *Ascococcus mesenterioides* following the classification of Cohn (12, p. 137). Van Tieghem (12, p. 464) gave the first adequate description of the organism, describing it as similar to the genus *Nostoc* of the green algae, and he gave it the designation which is still extensively used, namely, *Leuconostoc mesenterioides*.

[1] *Manuscript received July 31, 1931.*

Contribution from the laboratories of the Department of Bacteriology and the Department of Industrial and Cellulose Chemistry, McGill University, Montreal, with financial assistance from the National Research Council of Canada and the Canadian Pulp and Paper Association.

[2] *Graduate student, Department of Bacteriology, McGill University, and holder of a studentship under the National Research Council.*

[3] *Professor of Industrial and Cellulose Chemistry, McGill University.*

Béchamp (1) proposed the name "viscose" for the viscous products arising from the action of organized ferments on sucrose solutions, and stated that the gum formed was rarely homogeneous. The "viscose" which he examined yielded both glucose and dextrins on hydrolysis, as in the case of starch; in the case of the dextrins from "viscose" the hydrolysis took place more slowly. He agreed with Pasteur (16) that both "viscose" and mannitol were formed during fermentation, but obtained somewhat different proportions of these products. Since neither author describes the organism employed, nor the length of time of fermentation, no definite conclusions can be drawn from their work. It is of interest that Béchamp found that only sucrose was able to undergo the "viscous" fermentation, and that neither invert sugar, glucose, nor fructose could be transformed into "viscose." Däumichen (4) experimented with a mucilaginous material from a sugar factory and found that it resembled Scheibler's dextran in its physical and chemical properties.

Bräutigam (3) isolated an organism from a gelatinous digitalis infusion which he named *Micrococcus gelatinogenes*. This bacterium formed, from the sucrose present in the infusion, a product practically identical with Scheibler's dextran. Both glucose and fructose were found as by-products in the solution. Undoubtedly the *Micrococcus gummosus* isolated and described by Happ (7) was quite closely related to Bräutigam's micrococcus. This bacterium formed a viscous material in solutions containing sucrose or maltose, but Happ made no attempt to study the slime in detail.

Liesenberg and Zopf (13) were the first to isolate strains of *Leuconostoc mesenterioides* in pure culture form, and they succeeded in purifying them by virtue of their resistance to heat. They studied in detail three strains isolated from quite different sources, and their results led them to believe that no marked variation existed among them. In general the properties of these organisms were described by them as follows: When embedded in gelatinous masses they possess a marked resistance to heat, being able to withstand readily a temperature of 75° C. for 15 min. In suitable nutrient solutions which contained either sucrose or glucose, gigantic gelatinous capsules consisting of Scheibler's dextran are formed. The authors found that the gelatinous capsules arose only when the cultures were grown in suitable nutrient solutions containing sucrose or glucose, and that lactose, maltose or dextrin were unsuitable for polysaccharide formation. The strains studied were able to invert sucrose with the formation of fructose and glucose. The addition of relatively large amounts of chlorides (3-5% of calcium chloride, or 1-3% of sodium chloride) accelerated growth, and increased the amount of dextran formed.

Three types of *Leuconostoc* were isolated and described in some detail by Zettnow (19). One was named *Leuconostoc opalanitza*, and a second *Leuconostoc aller*, two variants of this latter organism being described. A detailed study of the cultural and morphological data submitted by Zettnow with reference to these strains appears to indicate that he was dealing merely with growth variants of the same organism, for the differences which he stresses are based merely on the rate and type of growth, as well as with variations in the appear-

ance of colonies of the organisms when grown on sucrose- or glucose-containing nutrient media.

Considerable differences were noted by Liesenberg and Zopf (13) and by Zettnow (19) in the different varieties of *Leuconostoc*. Thus the growth of the strains studied by the former authors was favored by the addition to the medium of fairly large amounts of sodium or calcium chloride, while the growth of the latter author's strains was definitely retarded by similar concentrations of the same salts. While gelatinous capsules corresponding to Scheibler's dextran were formed in media containing either sucrose or glucose by Liesenberg and Zopf's strains, in the case of Zettnow's strains this occurred only with sucrose. Peptone-free solutions, in which the source of nitrogen was asparagine, would not support the development of Zettnow's cultures, while Liesenberg and Zopf noted distinct, though somewhat poor development, of the species studied by them. Again, Zettnow observed that his strains showed definite growth after 24 hr. or somewhat longer, at 8-9° C., while the last-named author's cultures failed to exhibit any visible development at 9-11° C. Morphologically there was, apparently, no visible difference in the various strains studied by the above-named investigators. Probably one of the most significant differences found among the various species studied was that noticed by Liesenberg and Zopf in that the organisms investigated by them were able to withstand a temperature of 75° C. for 15 min. as well as prolonged desiccation, without losing their ability to produce the characteristic "froschlaich" formation in nutrient solutions containing sucrose. Zettnow's strains were exceedingly susceptible to heat and would not withstand drying for even six weeks.

Beijerinck (2) showed that many "dextran-forming" bacteria are merely lactic-acid-producing cocci, which form dextran as a substance of the cell wall. Moreover he showed that these organisms are by no means found occurring only in sucrose and related products, but are also present in natural earth, water, etc. He suggested the name *Lactococcus dextranicus* for this group of bacteria. His studies revealed the fact that only sucrose appears to yield dextran under the influence of these organisms.

Fernbach *et al.* (6) employed Beijerinck's *Lactococcus dextranicus* and noted that it formed a dextrosan only from sucrose, and not from invert sugar, nor from free fructose or glucose, thereby confirming Beijerinck's results.

Seiler (17) examined the slimes produced by several cultures of *Leuconostoc mesenterioides*, and observed that all of them yielded glucose and fructose upon hydrolysis. It is to be observed, in connection with this author's work, that, because of their relative insolubility in water, he did not purify the gums, and that they all contained a fairly large amount of both ash and nitrogen. It is not impossible that some of the unused sugars from the media employed in the preparation of the polysaccharides were mixed with them.

The most recent contribution to our knowledge of the genus *Leuconostoc* has been made by Hucker and Pederson (11), who have classified, mainly by virtue of the total amount of acid produced in solutions of a variety of carbo-

hydrates and polyhydric alcohols, three well-defined species, namely *L. mesenterioides*, *L. dextranicus* and *L. citrovorus*. In the light of this classification it is certain that the organism described by Liesenberg and Zopf (13), and by Zettnow (19), belonged to the species *mesenterioides*, while the type studied by Beijerinck (2), and later by Fernbach *et al.* (6), was evidently of the *dextranicus* species. The main criterion for the differentiation of *L. mesenterioides* and *L. dextranicus* according to Hucker and Pederson (11) is found in the fact that the former ferments the pentoses with the formation of acid, while the latter does not ferment the pentoses.

Lippmann (14) gives probably the most comprehensive summary of the literature dealing with the physical and chemical properties of the dextrans described by various authors. It is quite evident from his account that the various authors who studied these "dextrans" were by no means dealing with homogeneous products in so far as their physical and chemical properties were concerned. In no case has a really detailed study been made of the dextran polysaccharide formed by *L. mesenterioides*. This investigation was therefore undertaken with the intent of studying the conditions surrounding the formation of the dextran polysaccharide by *L. mesenterioides* and at the same time to prepare sufficient pure material for chemical investigations of the product.

Discussion of Results

Four strains of *Leuconostoc mesenterioides* have been studied. A brief survey of their morphological and cultural characteristics, in addition to their ability to form acid from various carbon compounds, indicated that two strains received from Holland were practically the same as the two strains received from Hucker (11), and which had been classified by him as typical strains of this species.

A series of experiments was performed, employing one of the two strains received from Holland, with the view of evolving a simple synthetic medium, and one most favorable for the production of dextran by the strains in question. The results of these experiments indicated that, while a reaction corresponding to pH 8.0 represented the optimum value for polysaccharide formation, this same formation also occurred at values of pH 6.0 to 8.4. A synthetic medium having the following composition appeared to give the best yield of the dextran polysaccharide: peptone, 0.1, sucrose, 10, KCl, 0.1 and $Na_2HPO_4 \cdot 12\ H_2O$, 0.2%. Dextran formation in such a medium apparently reached a maximum 10 days after inoculation.

Subsequent investigation showed that solutions containing sucrose underwent a pronounced "viscous" fermentation with all four strains, while the two cultures received from Holland produced a somewhat similar minor change in solutions containing glucose. When melezitose, raffinose, lactose, maltose, fructose, galactose, xylose or glycerol were employed in place of sucrose, no "viscous" fermentation was observed. When unit volumes of these experimental solutions were treated with five volumes of 95% ethyl alcohol, a pronounced precipitation occurred with all four strains in the nutrient solutions containing sucrose. Slight precipitation also occurred with the two

strains received from Holland in the case of the nutrient solutions containing glucose. Observation indicates that slime formation in the case of glucose is a somewhat transient factor, occurring only when the cultures are very active with respect to the formation of polysaccharide from sucrose.

These results can in general be reconciled with those obtained by Liesenberg and Zopf (13) and by Zettnow (19). The first-named authors worked with strains of *Leuconostoc* which were apparently much more resistant and more active than those studied by the last-named investigator, and they found that marked "dextran" formation took place in nutrient solutions containing either sucrose or glucose. Zettnow however apparently found that his cultures yielded "dextran" only with solutions containing sucrose. It seems probable that only very active cultures of *L. mesenterioides* are capable of forming "dextran" from glucose, although this cannot as yet be stated definitely.

No definite information of a correlation between the structure of "dextran" and the sugar sources which lend themselves to its formation has yet been obtained although the subject is under active investigation. Thus if it be assumed that "dextran" arises from the glucose portion of the sucrose molecule, as has been suggested by Fernbach *et al.* (6), in a manner analogous to the formation of levan from the fructofuranose residue in sucrose (8), then the glucopyranose portion of the melezitose molecule might be expected to give rise to the formation of dextran. However, experimental evidence has shown that this is not the case. Again, there is no apparent reason why these organisms should not be as active with respect to ordinary glucopyranose as they are towards sucrose. At the present time, therefore, it is impossible to state that there is any definite relation between the structure of "dextran" and the sugar source utilized in its synthesis.

Experimental

Culture Media Employed

(1) ***Beef infusion broth medium.*** Beef infusion broth* with the addition of 0.2% $Na_2HPO_4.12H_2O$ and 0.1% KCl. Reaction adjusted to pH 8.0 ± 0.1. (2) ***Sucrose beef infusion broth.*** Medium (1) with the addition of 10% of sucrose. Reaction adjusted to pH 8.0 ± 0.1. (3) ***Sucrose beef infusion gelatine.*** Medium (2) with the addition of 15% of gelatine (Bacto). Reaction adjusted to pH 8.0 ± 0.1. (4) ***Beef infusion gelatine.*** Medium (1) with the addition of 15% of gelatine (Bacto). Reaction adjusted to pH 8.0 ± 0.1. (5) ***Sucrose-broth.*** Sucrose, 10%; $Na_2HPO_4.12\ H_2O$, 0.2%; KCl, 0.5%; peptone, 0.1%; dissolved in distilled water. Reaction adjusted to pH 8.0 ± 0.1. (6) ***Sucrose-agar.*** Medium (5) with the addition of 1.5% of agar (Bacto). Reaction adjusted to pH 8.0 ± 0.1. (7) ***Litmus milk.*** Prepared according to the method advocated by the Society of American Bacteriologists (18).

All of the above media were sterilized for 20 min. at 15 lb. steam pressure.

**The beef infusion broth employed was prepared in the following manner: 500 gm. of chopped lean beef was boiled in tap water (one litre) for ½ hr. The mixture was then strained through cotton wool, and 1% of peptone and 0.5% of NaCl added to the filtrate. The solution was heated for 10 min. in flowing steam, and the pH adjusted to 7.6 by the addition of N/10 NaOH. Subsequently the solution was again heated for 20 min. in flowing steam, filtered through filter paper, and sterilized in an autoclave for 20 min. at 15 lb. steam pressure. The final medium had a reaction corresponding to pH 7.4 ± 0.1.*

Broth for the Determination of "Acid Production" from Various Carbon Compounds

Two solutions were prepared: (a) 1.0% peptone; 0.2% NaCl dissolved in distilled water and the pH adjusted to 6.8 ± 0.1. A 1.6% alcoholic solution (1 cc.) of brom cresol purple, per litre of solution, was added as indicator. (b) A 4% solution of the carbon compound under investigation dissolved in distilled water.

Five cc. quantities of solution (a) were placed in cotton plugged test tubes while solution (b) was placed in an Erlenmeyer flask. All solutions were sterilized for 20 min. at 15 lb. steam pressure. Subsequently they were cooled, and 5-cc. portions of solution (b) added to 5-cc. portions of solution (a), observing aseptic precautions. In this manner 2% solutions of a number of carbon compounds were prepared (see Table I).

Cultures Employed

Through the courtesy of Professor R. O. Herzog, Director of the Kaiser-Wilhelm Institute for Fibre Chemistry, Berlin-Dahlem, Germany, two strains of *Leuconostoc mesenterioides* were obtained from the Technische Hoogeschool Laboratorium voor Mikrobiologie, Delft, Holland. Throughout this investigation these strains have been designated as cultures 1 and 2. In order to ensure pure cultures for experimental work these strains were plated on sucrose-agar, and, after a suitable incubation period at 30° C., representative colonies were transferred to sucrose-broth, and the resulting cultures employed in subsequent experiments.

In addition, several strains of *L. mesenterioides* were obtained from the New York Agricultural Experiment Station through the kindness of Dr. G. J. Hucker. Two of the most active of these, as judged by their ability to form dextran polysaccharide from sucrose broth, were selected for experimental work. These have been designated in this report as cultures 3 and 4: culture 3 being identical with strain 60, and culture 4 with strain 5, as described by Hucker (11).

Throughout this investigation these cultures have been transferred at fairly regular intervals (2-5 days) in either sucrose beef infusion broth, or ordinary sucrose-broth, incubating the cultures at 30° C. In this manner they have been maintained in fairly active condition with reference to the production of "dextran" slime, although, for no apparent reason, the ability to form mucilaginous material from sucrose-broth has varied considerably from time to time, thus making experimental work somewhat difficult.

The only essential variation among the four strains studied appears to lie in their varying ability to produce acid from certain carbon compounds studied, although their morphological and cultural characteristics showed no significant variation. The following general description of the morphological and cultural characteristics is, therefore, applicable to all four of the strains studied.

Cultural Characteristics

Beef infusion broth. Slight turbidity; after approximately three days' incubation a white sediment settles to the bottom of the tube. ***Beef infusion***

gelatine stab culture. White, uniform, filiform growth along the line of inoculation; flat, white surface growth, no liquefaction of the gelatine. *Sucrose beef infusion broth.* Rapid development, the medium becomes distinctly opaque and viscous within 24 hr. inoculation, and usually gelatinous within five days of inoculation. After five days' incubation gas bubbles usually appear, and a cartilaginous layer develops at the bottom of the tube. *Sucrose beef infusion gelatine stab.* A profuse, uniform, white, irregular, stalactite-like growth develops along the line of inoculation, and a raised cartilaginous knob forms at the surface. The medium is split by gas formation after about five days. *Sucrose beef infusion gelatine slope culture.* Very abundant, white, raised, irregular, cartilaginous surface growth, which appears to sink into the medium, although the gelatine is not liquefied. *Sucrose-broth.* The development is somewhat slower than in sucrose beef infusion broth. Within 48 hr. of inoculation the medium becomes slightly opalescent and viscous, and, as a rule, becomes distinctly gelatinous within five days of inoculation. *Litmus milk.* Slowly becomes acid with the formation of a very weak clot, and slight, transient reduction of the litmus.

Morphological Characteristics

Gram positive cocci; usually exist as diplococci, although short chains of rarely more than six members are frequently formed. The diameter varies between about 0.5 to 1.0 μ, according to the strain and the medium upon which the cells have been cultivated. Capsules have never been observed, even in very young sucrose-broth cultures, when the Gin method of staining (18) was employed. The slime appears generally to be evenly diffused, and in this respect these strains are, apparently, different from those described by Liesenberg and Zopf (13) and by Zettnow (19), who stated that the organisms studied by them formed huge gelatinous capsules in nutrient media containing sucrose. It is not improbable that the ability of organisms of this genus to form capsules may vary considerably, and that prolonged cultivation under artificial laboratory conditions may involve a transient, or even permanent disappearance of this power.

Production of Acid from Various Carbon Compounds

A study of the ability of the four cultures under investigation to form acid from a variety of carbon compounds was made, chiefly in order to compare the strains received from Holland with those received from Hucker (11) in regard to this power.

Duplicate 10-cc. portions of solutions of the carbon compounds under investigation (see Table I) were inoculated with one 2-mm. loopful of a 48-hour-old sucrose-broth culture of the organism under investigation. Subsequently all the solutions were incubated at 30° C. for 10 days, and the total amount of acid present in each then determined by titrating with $N/10$ NaOH until the original purple color of the indicator returned.

The results of this experiment, which are recorded in Table I, show that all four cultures are very similar in regard to their fermentative power, especially with reference to their ability to form acid from the pentoses (arabinose and

xylose). Thus the two strains received from Holland (cultures 1 and 2) may be considered as typical strains of *Leuconostoc mesenterioides* according to Hucker (11), in regard to their morphology, fermentative power, and cultural characteristics.

TABLE I

AMOUNT OF SODIUM HYDROXIDE REQUIRED TO NEUTRALIZE THE ACID PRODUCED IN 10-CC. PORTIONS OF 2% SOLUTIONS OF THE CARBON COMPOUNDS INDICATED

Carbon compound	No. of cc. of $N/10$ NaOH required to neutralize the acid present in solutions of cultures								Carbon compound	No. of cc. of $N/10$ NaOH required to neutralize the acid present in solutions of cultures							
	1		2		3		4			1		2		3		4	
	(a)*	(b)*	(a)	(b)	(a)	(b)	(a)	(b)		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Starch	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Fructose	1.1	1.15	1.6	1.6	0.1	0.15	0.1	0.1
Dextrin	0.1	0.1	0.05	0.05	0.0	0.0	0.0	0.0	Arabinose	0.9	1.0	1.3	1.0	1.4	1.6	1.3	1.3
Inulin	0.0	0.0	0.0	0.0	1.3	1.4	2.3	2.0	Xylose	1.0	0.95	2.5	2.1	1.9	2.2	1.3	1.5
Raffinose	0.25	0.25	0.0	0.0	0.0	0.0	0.0	0.0	Ethylene								
Sucrose	1.0	0.9	1.8	1.8	1.3	1.3	1.5	1.6	glycol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maltose	0.5	0.4	1.2	1.2	0.8	1.0	0.9	1.0	Glycerol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lactose	0.4	0.4	0.0	0.0	0.4	0.4	0.2	0.3	Erythritol	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Glucose	0.7	0.7	1.55	1.45	0.7	0.8	1.4	1.7	Mannitol	0.0	0.0	1.7	1.9	0.15	0.15	0.2	0.25
Galactose	0.35	0.35	0.5	0.4	0.3	0.4	0.3	0.3	Controls	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

(a) and (b) represent duplicate experiments.

Optimum Conditions for the Production of "Dextran" by Leuconostoc mesenterioides

Owen (15) determined the optimum conditions for the formation of levan by the soil group of bacilli, especially *Bacillus vulgatus*. No author seems to have studied thoroughly the conditions surrounding the production of "dextran" by *L. mesenterioides*. Liesenberg and Zopf (13) noted that the strains of *L. mesenterioides* studied by them apparently exhibited maximum "dextran" formation in an alkaline medium, and it therefore appeared essential to determine first the optimum pH for the formation of polysaccharide by this organism, and to carry out experiments to obtain a simple synthetic medium for the production of dextran for chemical investigations. Culture 1 was employed throughout this series of experiments.

Experiment I. Optimum pH for the Production of Dextran from Sucrose

A solution having the following composition was prepared: sucrose, 10%; KH_2PO_4, 0.5%; NaCl, 0.5%; and peptone, 0.5%; dissolved in distilled water. Quantities (30 cc.) of this solution were brought to various pH values by the addition of $N/1$ NaOH employing the colorimetric method. The approximate amount of $N/1$ NaOH required in each instance to bring the solution to the required pH, together with the indicator employed, is given in Table II.

The solutions were all sterilized separately by filtering through a Berkefeld "W" filter employing suction (500 mm.), and 25 cc. of each was placed in a sterile, cotton-plugged 50-cc. Erlenmeyer flask. The solutions were incubated at 37.5° C. for 48 hr., and at the conclusion of this time showed no visible signs

TABLE II

AMOUNT OF $N/1$ NaOH REQUIRED TO BRING 30 CC. OF THE 10% SUCROSE SOLUTION TO THE pH VALUES INDICATED

Indicator	Brom thymol blue								Phenol red				
Cc. $N/1$ NaOH added (approx.)	0.1	0.3	0.5	0.6	0.7	0.8	0.85	0.9	0.95	1.0	1.05	1.1	1.15
pH of soln. (colorimetric)	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4

of contamination. Each solution was then inoculated with one 2-mm. loopful of a 48-hour-old sucrose-broth culture of *L. mesenterioides*, and all were incubated at 30° C.

Determinations of the amount of dextran present were made at intervals employing the following technique: 5 cc. of the reacting sucrose solution was withdrawn from the culture flask under aseptic conditions, and placed in a weighed 100-cc. beaker. This solution was made just alkaline to phenolphthalein by the addition of from one to three drops of $N/1$ NaOH, in order to have uniform conditions for precipitation. Ethyl alcohol (95%, 25 cc.) was then added to each beaker, and the dextran permitted to precipitate by allowing the beaker and its contents to stand (covered) at room temperature for 12 hr. The clear, supernatant liquor was carefully decanted from the dextran which adhered firmly to the glass, and the beaker and its contents placed in a vacuum desiccator over $CaCl_2$ and P_2O_5 for 12 hr.; the beaker was then weighed, and the weight of the precipitate calculated by difference. The results of this experiment, recorded in Table III, indicate that dextran can be formed by this organism in both acid and alkaline solution. The optimum reaction, as judged by the greatest amount of polysaccharide formed after ten days' incubation, is at pH 8.0. It is of interest that, while more dextran is formed at pH 6.8 to 7.2 after 2 days' incubation, after ten days' incubation much more of this polysaccharide is obtained from solutions with a pH of about 8.0.

TABLE III

AMOUNT OF DEXTRAN FORMED BY *L. mesenterioides* IN 10% NUTRIENT SUCROSE SOLUTIONS OF VARYING pH

pH of solution	Weight of crude dextran in grams after:			pH of solution	Weight of crude dextran in grams after:		
	2 days	4 days	10 days		2 days	4 days	10 days
6.0	0.0409	0.0436	0.0628	7.4	0.0685	0.0938	0.1410
6.2	0.0857	0.1031	0.1021	7.6	0.0544	0.1166	0.1421
6.4	0.0740	0.0940	0.1065	7.8	0.0586	0.1188	0.1758
6.6	0.0730	0.1071	0.1245	8.0	0.0641	0.1043	0.2173
6.8	0.0831	0.0939	0.1316	8.2	0.0515	0.0913	0.1496
7.0	0.0857	0.0930	0.1391	8.4	0.0513	0.0563	0.1126
7.2	0.0827	0.1052	0.1397				

Experiment II. Optimum Concentration of Peptone for the Formation of Dextran from Sucrose

Portions (30 cc.) of aqueous solutions containing 10% sucrose, 0.2% $Na_2HPO_4 \cdot 12\ H_2O$; 0.5% KCl and varying concentrations (1.0, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 and 0.0%) of peptone (Bacto) were prepared and the reaction adjusted to pH 8.0 ± 0.1. In all the succeeding experiments the pH of the solutions was brought to the same value and the subsequent treatment was as outlined under experiment 1.

The results (Table IV) show that the optimum concentration of peptone is about 0.05 to 0.1%, and that lower, or higher concentrations, respectively, give lower yields of the polysaccharide.

TABLE IV
EFFECT OF VARYING CONCENTRATIONS OF PEPTONE ON THE AMOUNT OF DEXTRAN FORMED IN TEN PER CENT SUCROSE SOLUTIONS

Percentage of peptone in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:			Percentage of peptone in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:		
	3 days	8 days	30 days		3 days	8 days	30 days
1.0	0.0949	0.0914	0.0857	0.01	0.0092	0.0577	0.0603
0.5	0.0813	0.1067	0.1368	0.005	0.0120	0.0329	0.0547
0.1	0.0793	0.1011	0.1361	0.001	0.0093	0.0323	0.0162
0.05	0.0742	0.1333	0.1414	0.000	0.0112	0.0250	0.0133

Experiment III. Optimum Concentration of Sucrose for the Formation of Dextran from this Sugar (pH 8.0)

Aqueous solutions (60 cc.) were used containing 0.1% peptone, 0.2% $Na_2HPO_4 \cdot 12\ H_2O$, 0.5% KCl, and varying concentrations of sucrose (20, 10, 5, 2 and 1%). The results (Table V) indicate that the higher the concentration of sucrose, up to 20%, the greater the amount of dextran formed. However, the yield of dextran per unit amount of sucrose was greater in the lower than in the higher concentrations. For convenience in experimental work a 10% solution can be employed advantageously, thus avoiding the difficulty occasioned by the presence of sucrose in the "alcohol-precipitated product" employed for chemical investigations.

TABLE V
AMOUNT OF DEXTRAN PRODUCED BY *L. mesenterioides* FROM SOLUTIONS CONTAINING VARYING AMOUNTS OF SUCROSE

Percentage of sucrose in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:				
	3 days	7 days	14 days	21 days	28 days
20	0.0656	0.0724	0.0881	0.1873	0.2141
15	0.0263	0.0333	0.0787	0.1011	0.1882
10	0.0242	0.0320	0.0651	0.0945	0.1453
5	0.0139	0.0254	0.0448	0.0485	0.0942
2	0.0092	0.0101	0.0322	0.0395	0.0397
1	0.0075	0.0099	0.0123	0.0182	0.0236

Experiment IV. Optimum Concentration of $Na_2HPO_4.12H_2O$ for the Formation of Dextran from Sucrose (pH 8.0)

The solutions (60 cc.) used contained 0.1% peptone; 0.5% KCl; 10% sucrose; and varying concentrations of $Na_2HPO_4.12H_2O$ (1.0, 0.5, 0.1, 0.05, 0.01 and 0.00%). The results, recorded in Table VI, show that the optimum concentration of $Na_2HPO_4.12H_2O$ lies between 0.5 and 0.1% at the twenty-

TABLE VI

EFFECT OF VARYING CONCENTRATIONS OF $Na_2HPO_4.12H_2O$ ON THE AMOUNT OF DEXTRAN FORMED FROM SUCROSE SOLUTIONS BY *L. mesenterioides*

Percentage of $Na_2HPO_4.12H_2O$ in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:				
	3 days	7 days	14 days	21 days	28 days
1.0	0.0608	0.0556	0.0456	0.0983	0.2228
0.5	0.0282	0.0354	0.0883	0.1137	0.1419
0.1	0.0261	0.0249	0.1104	0.1481	0.1276
0.05	0.0162	0.0200	0.0742	0.0868	0.1002
0.01	0.0149	0.0183	0.0608	0.0719	0.0892
0.00	0.0144	0.0181	0.0394	0.0656	0.0810

first day of incubation, and that at longer time intervals higher concentrations of this salt cause an increase in the amount of polysaccharide formed. Presumably 1% of $Na_2HPO_4.12H_2O$ would give higher yields of dextran on prolonged incubation, but for experimental work, especially for the production of a product low in ash for the chemical investigations, a concentration of 0.2% should prove quite satisfactory.

Experiment V. Optimum Concentration of KCl for the Formation of Dextran from Sucrose

Quantities (60 cc.) of the following solutions containing 0.1% peptone, 10% sucrose, 0.2% $Na_2HPO_4.12H_2O$ and varying concentrations of KCl (1.0, 0.5, 0.1, 0.05, 0.01, and 0.00%) were employed. The results (Table VII) show that, in the early stages of the formation of this polysaccharide, a concentration of 1% of KCl gives the highest yield, while on prolonged incubation the amounts of dextran formed in the solutions containing lower concentrations of this salt become greater, so that finally a concentration of 0.1% KCl appears to be the optimum value. For experimental work a concentration of 0.1% should prove satisfactory, having regard to the isolation of a product readily freed from ash.

The results of the above five experiments, when taken collectively, indicate that a medium having the following composition should prove satisfactory for the experimental production of dextran by *L. mesenterioides:* 10% sucrose, 0.1% peptone, 0.2% $Na_2HPO_4.12\ H_2O$ and 0.1% KCl, the reaction of such a medium being adjusted to pH 8.0 ± 0.1. In view of the fact that good yields of dextran from sucrose could be obtained by the use of the above medium it was not considered necessary to determine the possible increase in the same which might result due to the addition of other inorganic salts

TABLE VII

EFFECT OF VARYING CONCENTRATIONS OF KCl ON THE AMOUNT OF DEXTRAN FORMED BY *L. mesenterioides*

Percentage of KCl in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:				
	3 days	7 days	14 days	21 days	28 days
1.0	0.0862	0.0903	0.1195	0.1473	0.1626
0.5	0.0771	0.0831	0.1401	0.1493	0.1924
0.1	0.0339	0.0383	0.1201	0.1290	0.2195
0.05	0.0332	0.0397	0.1183	0.1246	0.1512
0.01	0.0295	0.0304	0.0512	0.0660	0.0894
0.00	0.0148	0.0237	0.0397	0.0351	0.0315

(magnesium, calcium, etc.), especially since the addition of such salts might make it more difficult to isolate a product practically free from ash for chemical studies.

Experiment VI. Time Required for Maximum Dextran Formation in Sucrose Solutions

The following solutions were prepared: Solution (a). 20% sucrose in distilled water. Solution (b). 0.2% peptone; 0.2% KCl; 0.4% $Na_2HPO_4 \cdot 12H_2O$; dissolved in distilled water, and the reaction adjusted to pH 8.0 ± 0.1.

Two 125-cc. quantities of the above solutions were placed in 300-cc. Florence flasks, sterilized in an autoclave at 15 lb. steam pressure for 20 min., cooled, and mixed, observing aseptic precautions. After inoculation (Culture 1) and incubation, the dextran formed was determined. The results (Table VIII) may be interpreted as showing that dextran formation does not increase after the tenth day of incubation.

TABLE VIII

RELATION BETWEEN TIME AND THE AMOUNT OF DEXTRAN FORMED IN 10% SUCROSE SOLUTIONS

Incubation period in days	Weight of crude dextran in grams per 5 cc. of medium in solution		Incubation period in days	Weight of crude dextran in grams per 5 cc. of medium in solution	
	1	2		1	2
0	0.0030	0.0035	10	0.4110	0.3989
2	0.0519	0.0575	13	0.4105	0.4067
4	0.1611	0.1701	15	0.3888	0.3901
7	0.3607	0.3544	21	0.4131	0.4182
8	0.3789	0.3806	24	0.4010	0.4005
9	0.3765	0.3826	30	0.3981	0.4109

STUDY OF THE SUGARS SUITABLE FOR THE FORMATION OF DEXTRAN BY LEUCONOSTOC MESENTERIOIDES

In view of the fact that levan-forming bacilli were found to exhibit great specificity for the terminal fructofuranose group in sucrose and raffinose (8), experiments were next instituted to ascertain whether or not the strains of

L. mesenterioides under investigation were capable of exerting a selective action only with sugars containing specific sugar residues.

Experiment VII. Polymerizing Action of Four Strains of L. mesenterioides with Reference to the Sugar Involved (pH of solutions = 8 0 ± 0.1)

The solutions used contained 10% of the carbon compound under investigation, together with 0.1% peptone, 0.1% KCl and 0.2% $Na_2HPO_4 \cdot 12\ H_2O$. After sterilizing, employing suction, the solutions were checked by incubating for three days at 30°C. Five separate "sets" representing ten different carbon compounds were prepared. One set was retained as a control, and each of the four remaining sets was inoculated separately from a 48-hour-old sucrose-broth culture of one of the four strains of *L. mesenterioides* being studied. After incubation for 10 days at 30° C., the dextran in each solution was determined as follows:

Ethyl alcohol (95%, 50 cc.) was placed in a weighed 100-cc. beaker, and 10 cc. of the culture fluid added drop by drop. The beakers were covered and the solutions permitted to stand for 12 hr. at room temperature to allow the dextran to settle. The clear, supernatant, alcoholic solution was carefully decanted to avoid disturbing the precipitates, the beakers inverted for 15 min. to remove all the remaining alcoholic solution, and finally the beakers and their contents dried thoroughly by placing in a vacuum desiccator over $CaCl_2$ and P_2O_5 for 24 hr. The results are given in Table IX.

In the case of all four strains the sucrose solutions became distinctly viscous after approximately three days' incubation, and distinctly mucilaginous after ten days' incubation. The solutions containing glucose became slightly viscous in the case of cultures 1 and 2, and apparently remained unchanged with cultures 3 and 4. The weights given in Table IX indicate that dextran is formed in large amounts from sucrose by all four strains, and in small amounts from glucose by cultures 1 and 2. The weights obtained in the case of carbon com-

TABLE IX

DEXTRAN PRODUCTION FROM VARIOUS CARBON COMPOUNDS BY FOUR STRAINS OF *Leuconostoc mesenterioides*

Carbon compound	Weight of dextran in grams per 10 cc. of culture medium in:				
	Culture 1	Culture 2	Culture 3	Culture 4	Controls*
Raffinose	0.0047	0.0056	0.0037	0.0033	0.0035
Melezitose	0.0048	0.0064	0.0053	0.0051	0.0052
Sucrose	0.5051	0.4769	0.2440	0.4549	0.0052
Maltose	0.0043	0.0053	0.0042	0.0033	0.0032
Lactose	0.0039	0.0051	0.0056	0.0041	0.0041
Glucose	0.0257	0.0233	0.0047	0.0039	0.0044
Fructose	0.0056	0.0071	0.0038	0.0027	0.0028
Galactose	0.0061	0.0063	0.0083	0.0060	0.0055
Xylose	0.0057	0.0053	0.0051	0.0045	0.0029
Glycerol	0.0063	0.0049	0.0032	0.0043	0.0041

**The control in each instance represents the residue due to the sugar adhering to the container in the case of the uninoculated controls, which were treated similarly, in other respects, to the inoculated solutions.*

pounds other than glucose and sucrose cannot be considered significant when compared with the weights obtained in the control solutions. No formation of a viscous solution took place in solutions containing sugars other than sucrose or glucose.

Preparation of Dextran from Sucrose for Chemical Investigation

The following solutions are prepared: Solution (a). 20% sucrose in distilled water adjusted to pH 7.0 by adding $N/10$ NaOH. Solution (b). 0.2% peptone; 0.4% $Na_2HPO_4 . 12\ H_2O$ and 0.2% KCl in distilled water, the reaction being adjusted to pH 8.0 ± 0.1.

The above solutions are sterilized separately in one-litre Erlenmeyer flasks in 500-cc. quantities in the autoclave for 20 min. at 15 lb. steam pressure. The resulting solutions are then cooled, mixed carefully under aseptic conditions, inoculated with one 2-mm. loopful of a 48-hour-old sucrose-broth culture of the strain *of L. mesenterioides* under investigation, and incubated for ten days at 30° C. The yield of anhydrous, practically ash-free, pure polysaccharide, calculated on the net weight of sucrose taken varied between 25-35%. The ash content in the purest specimens did not amount to more than 0.05%. The method of purification is to be described in a future paper by Hibbert and coworkers dealing with the chemical structure of dextran, and its application in the study of immunological problems.

References

1. Béchamp, A. Compt. rend. 93:78-82. 1881.
2. Beijerinck, M. W. Folia Mikrobiol. 1: 377. 1912.

2a. Beijerinck, M. W. Zentr. Biochem. Biophys. 14: 679. 1913. (Abstract of Ref. 2).

3. Bräutigam, W. Chem. Centr. 63: 648. 1892.
4. Däumichen, P. Chem. Centr. 61: 1000. 1890.
5. Durin, E. Compt. rend. 83: 128-131. 1876.
6. Fernbach, A., Schoen, M. and Hagiwara, S. Compt. rend. soc. biol. 92: 1418-1419. 1925.
7. Happ, C. Centr. Bakt. Parasitenk. 1 Abt. 14: 175-177. 1893.
8. Harrison, F. C., Tarr, H. L. A. and Hibbert, H. Can. J. Research, 3: 449-463. 1930.
9. Hibbert, H., and Brauns, F. Can. J. Research, 4: 596-604. 1931.
10. Hibbert, H., Tipson, R. S. and Brauns, F. Can. J. Research, 4: 221-239. 1931.
11. Hucker, G. J. and Pederson, C. S. N.Y. State Agr. Exptl. Stn. Tech. Bull. 167. 1930.
12. Lafar, F. Handbuch der Technischen Mykologie, Bd. 1 and 2, Jena. 1904-7.
13. Liesenberg, C. and Zopf, W. Centr. Bakt. Parasitenk. 12: 659-661. 1892.
14. Lippmann, E. O. von. Die Chemie der Zuckerarten, 3d ed. Braunschweig. 427-430. 1904.
15. Owen, W. L. La. Sugar Exptl. Stn. Bull. 125. 1911.
16. Pasteur, L. Bull. soc. chim. p. 30. 1861.
17. Seiler, F. Z. Untersuch. Nahrungs. Genussmittel. 2: 513-528. 1905.
18. Society of American Bacteriologists. Committee on bacteriologic technic. Manual of methods for pure culture study of bacteria. 1926.
19. Zettnow, E. Z. Hyg. 57: 154-172. 1907.

STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES.

XXXVIII. PREPARATION, SEPARATION AND IDENTIFICATION OF THE ISOMERIC BROMOETHYLIDENE GLYCEROLS[1]

By Harold Hibbert[2] and Earl Hallonquist[3]

Abstract

Additional evidence is given regarding the influence of polar radicals, or atoms, on the ease and nature of acetal condensations involving glycerol.

Bromoethylidene glycerol has been prepared as a mixture of the isomeric five- and six-membered ring forms, by the condensation of bromoacetaldehyde with glycerol. This mixture was separated into the two isomeric forms by taking advantage of differences in the physical properties of their benzoates.

The equilibrium ratio of six- to five-membered acetal at room temperature as obtained in the above preparation was found to be 1:15. Under the influence of 1% dry HCl at 25° C. the ratio changed to 1: 8.

By conversion of the acetals into the methyl ethers, and comparison of these with the corresponding products of known constitution obtained by condensation of bromoacetaldehyde with glycerol α- and β-methyl ethers respectively, the identity of each of the acetals was established.

A detailed description is given for the preparation of dibromoparacetaldehyde both in the crystalline form (50% yield) and as a crude syrup (75% yield).

Introduction

Hibbert and Hill (3) in 1923 pointed out for the first time that "the condensation of glycerol (1 mol) with an aldehyde (1 mol) represents an 'intramolecular partition,' and it seems logical to assume that in these reactions two isomers (namely a five- and a six-membered cyclic acetal) will be formed in each case, the relative amounts being dependent on the molecular configuration of the aldehyde employed."

Since that time acetal condensations of glycerol with formaldehyde (1), acetaldehyde (9), trimethylacetaldehyde (11), benzaldehyde (10), *p*-nitrobenzaldehyde (2), cinnamic aldehyde (7), chloral (5) and acetone (4) have been carried out in this laboratory. In all except the latter two cases, both the five- and six-membered ring structures were isolated. In the case of chloral and acetone, only the five-membered acetal could be identified.

In the present investigation bromoethylidene glycerol, formed by condensation of bromoacetaldehyde with glycerol, has been shown to be a mixture of both isomeric forms. The ratio of six-membered to five-membered acetal was found to be 1 : 15.

In a recent paper Hibbert, Morazain and Paquet (5), in discussing the nature of the factors involved in the mechanism of cyclic acetal formation, drew attention to the fact that with a series of alkyl and halogen substituted acetaldehydes, such as the following:

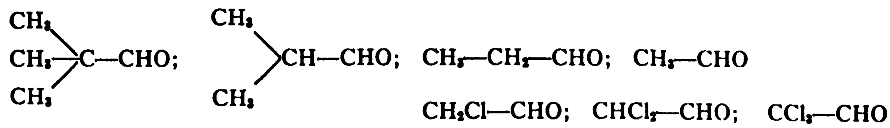
[1] *Manuscript received Sept. 16, 1931.*

Contribution from the Industrial and Cellulose Chemistry Laboratories, McGill University, Montreal, Canada, with financial assistance from the Canadian Pulp and Paper Association.

[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*

[3] *Postgraduate student, McGill University, and holder of a bursary under the National Research Council of Canada.*

Increase in ease of "half-acetal" formation →



← Increase in ease of cyclic acetal formation

it is to be expected according to Michael's theory that, as the polar character of the carbonyl (CO) group becomes more negative, the tendency towards addition of water, alcohols and polyhydroxy compounds will increase, the relative stability of the half-acetal formed will increase, and, since the half-acetal is an intermediate product, the relative ease of ring formation will decrease. These authors also suggested that the proportion in which five- and six-membered rings are formed from acetal condensations may be influenced by the polar character of the radicals present. These views were supported by the fact that condensation of trimethylacetaldehyde with glycerol takes place readily and in the absence of a catalyst (11), while with chloral, condensation takes place only under the influence of a strong dehydrating agent such as sulphuric acid or zinc chloride (5). Further, the proportion of six-membered acetal in the case of tertiary amylidene glycerol is relatively high, while it has not been possible to isolate any six-membered cyclic acetal in the case of trichloroethylidene glycerol.

Additional evidence is furnished by the data obtained in the present investigation on bromoethylidene glycerol, as can be seen readily from Table I.

Further work is being carried out in this laboratory with a view to obtaining data on the nature of the products formed from the condensation of isobutyraldehyde, propionaldehyde, dibromoacetaldehyde and bromal with glycerol.

TABLE I

YIELD AND PROPORTION OF ISOMERIC ALKYLIDENE GLYCEROL ACETALS FORMED FROM VARIOUS ALIPHATIC ALDEHYDES

Nature of aldehyde used	Yield of mixed acetals, %	Ratio of isomers at room temperature
$(CH_3)_3C$—CHO	65	1: 2
$(CH_3)_2CH$—CHO		
CH_3—CH_2—CHO		
CH_3—CHO	63	1: 4
CH_2Br—CHO	35	1: 15
$CHBr_2$—CHO		
CCl_3CHO	20	No six-membered isomer formed.

NOTE:—*Data for tertiary amylidene glycerol, ethylidene glycerol, and trichloroethylidene glycerol are taken from references (11), (9), and (5) respectively.*

The ratio indicated in each case is the proportion of six- to five-membered isomer.

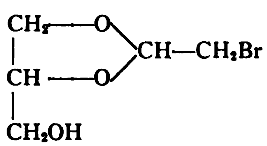
The Bromoethylidene Glycerols

A satisfactory method for the preparation of bromoacetaldehyde, which was required in large quantities for the preparation of crude bromoethylidene glycerol, was de-

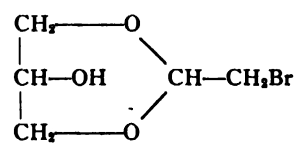
veloped only after considerable experimentation. This method gave constant yields of bromoparaldehyde, either in the form of a crude syrup, which was convenient to use for the preparation of bromoethylidene glycerol, or as pure crystalline dibromoparaldehyde. Bromoethylidene glycerol was prepared according to the method of Hibbert and Hill (3) by condensing bromoparaldehyde with glycerol in the presence of a small amount of sulphuric acid. The mixture of bromoethylidene glycerols so prepared was converted by the action of benzoyl chloride and pyridine into the corresponding benzoates. A crystalline and liquid benzoate resulted and these were separated and purified. The crystalline benzoate, on hydrolysis to the acetal, followed by methylation, gave a methyl ether having properties identical with those of the methyl ether prepared by the condensation of bromoparaldehyde with glycerol β-methyl ether. Similarly the liquid benzoate, on hydrolysis to the acetal, followed by methylation gave a product identical with that obtained by condensation of bromoparaldehyde with glycerol α-methyl ether.

As the bromoethylidene glycerol methyl ether synthesized from glycerol α-methyl ether can only have a five-membered ring, and that from glycerol β-methyl ether a six-membered ring, and since the two compounds differ sufficiently in physical properties, they serve as a means of identifying the five- and six-membered bromoethylidene glycerols respectively, when the latter are converted by methylation to their respective methyl ethers.

The structure of the two isomeric acetals must therefore be as follows:



1: 2-bromoethylidene glycerol.



1: 3-bromoethylidene glycerol.

Table II will serve to compare some physical constants of the acetals and their derivatives.

The slightly higher values for refractive index and density in the case of the 1:2-bromoethylidene glycerol 3-methyl ether obtained by methylation of 1:2-bromoethylidene glycerol, as compared with the values for the 1:2-bromoethylidene glycerol 3-methyl ether prepared synthetically from glycerol α-methyl ether, are presumably due to the fact that the former contained a small amount of 1:3-bromoethylidene glycerol 2-methyl ether. This impurity would raise slightly the values for refractive index, etc. The isolation in the pure state of the five- and six-membered bromoethylidene glycerols is rendered difficult on account of the impossibility of effecting a complete separation of the benzoates. While it is possible to obtain the solid benzoate (6-membered) perfectly pure, it was not found possible to free the liquid benzoate (five-membered) completely from all traces of the solid six-membered isomeric benzoate.

In general the relative values of the physical properties for the respective acetals and methyl ethers follow the trend observed in the case of the ethylidene

TABLE II

PROPERTIES OF THE BROMOETHYLIDENE GLYCEROLS AND DERIVATIVES

Compound	B.p., °C.	M.p., °C.	n_D^{25}	d_4^{25}
1:2-bromoethylidene glycerol	150-151/23 mm.		1.5008	1.6437
1:3-bromoethylidene glycerol	144-145/22 mm.		1.5067	1.6557
1:2-bromoethylidene glycerol 3-benzoate	170/0.6 mm.		1.5452	1.4618
1:3-bromoethylidene glycerol 2-benzoate		109		
1:2-bromoethylidene glycerol 3-methyl ether, prepared from the benzoate	117.5-119/22 mm.		1.4720	1.4694
1:2-bromoethylidene glycerol 3-methyl ether, synthesized from glycerol α-methyl ether	117-119/23 mm.		1.4708	1.4663
1:3-bromoethylidene glycerol 2-methyl ether, prepared from the benzoate	128-129/21 mm.		1.4790	1.4973
1:3-bromoethylidene glycerol 2-methyl ether, synthesized from glycerol β-methyl ether	127-129/21 mm.		1.4792	1.4963

glycerols (9). Thus the 1:3-acetal has a lower boiling point, higher refractive index and higher density than the 1:2-acetal, and the 1:3-methyl ether has a higher boiling point, higher refractive index and higher density than the 1:2-methyl ether, although the differences are not as great as in the cases of most other cyclic acetals.

Experimental

Preparation of Bromoacetaldehyde (Bromoparaldehyde)

In spite of numerous attempts it was found impossible to duplicate the method previously outlined by Stepanow *et al.* (12). The following procedure, however, gave satisfactory results.

Pure, freshly distilled paraldehyde (105 gm.) was placed in a three-necked flask fitted with mercury seal stirrer, dropping funnel and calcium chloride drying tube. Bromine (300 gm.), dried over concentrated sulphuric acid and distilled, was added drop by drop through the separatory funnel, the contents of the flask being stirred vigorously and maintained at −15° C. by immersion in a cooling bath. The addition of the bromine took about six hours. The mixture was then stirred for about five hours at −15° C. At the end of this time 150 gm. of sodium acetate, finely divided, and in the form of an aqueous sludge, was very slowly added, the temperature being kept below 0° C. The mixture was stirred for a few minutes and then allowed to stand at −10° C. for 10 hr. Enough water was added to dissolve the excess of sodium salts and the mixture was extracted four times with ether. The ether solution was placed in a large flask and stirred with an electrically driven stirrer, the temperature being maintained at −10° C. by immersion in the cooling bath. Sodium hydroxide (5%) was slowly added till the mixture was neutral to litmus.

The ether solution, which previously had been slightly brown in color, was now perfectly colorless. The ether layer was washed free from alkali with a small amount of water, further washed with concentrated sodium bisulphite solution, then again with water and finally dried over anhydrous sodium sulphate. The ether was distilled off under reduced pressure (water-pump). A very light straw-colored syrup (180 gm.) remained, representing a yield of 75% of crude product. This syrup, on standing overnight, crystallized. By filtering with suction, and recrystallizing the solid from warm alcohol, 120 gm. of pure dibromoparaldehyde (m.p. 57° C.) was obtained. Yield, 50%. This is the product prepared with so much difficulty by Stepanow *et al.* (12). It was found, however, to be more convenient to use the crude syrup in the preparation of bromoethylidene glycerol, and better yields were obtained than with the pure crystalline dibromoparaldehyde.

Preparation of Crude Bromoethylidene Glycerol

Crude syrupy bromoparaldehyde (170 gm.) and 128 gm. of pure glycerol were placed in a round-bottomed flask equipped with a mechanical stirrer. Sulphuric acid (40%, 15 drops) was added, and the contents of the flask slowly heated on the steam bath with good stirring. No appreciable reaction seemed to take place until the temperature reached 80° C. The reaction mixture was stirred at 80-90° C. for 20 hr., a slight amount of discoloration taking place. The mixture was extracted with ether, the ethereal solution neutralized with dilute sodium hydroxide solution, washed with a small amount of sodium bisulphite solution, then with water, and dried over anhydrous sodium sulphate. The ether was distilled off under reduced pressure and the remaining liquid fractionated under reduced pressure, yielding 111 gm. of substance, b.p. 145-155° C./22 mm. On refractionation 95 gm. of a pure bromoethylidene glycerol, b.p. 142-144° C./17 mm., was obtained (bath temp. 175-180° C.). Yield, 35% on basis of glycerol used.

Preparation of Benzoates of Bromoethylidene Glycerol

Dry HCl gas was bubbled into 251 gm. of bromoethylidene glycerol (prepared as above) until 2 gm. had been absorbed. The mixture was allowed to stand for 48 hr. at 25° C. It was then dissolved in 100 gm. of dry pyridine, and this solution added slowly, and with cooling, to a solution of 178 gm. of benzoyl chloride in 120 gm. of pyridine. The resulting reaction mixture was allowed to stand overnight at 40° C. It was then dissolved in ether, the ether solution washed several times with 1% aqueous sulphuric acid, then with dilute sodium bicarbonate solution, and finally with water. The ether extract was dried over anhydrous sodium sulphate, and the ether recovered by distillation. The residual, very viscous oil amounted to 336 gm., representing a yield of 87% of crude product. This viscous oil exhibited no tendency to crystallize when cooled to a low temperature, even on standing for several weeks, but at room temperature it began to crystallize and soon became a thick paste. All attempts to isolate the crystalline part of this paste by fractional precipitation from solvents failed. In all accessible solvents the difference in solubility between the solid and liquid benzoate was so slight, or

else the proportion of solid to oil was so small, that no separation could be made. Ligroin, alcohol, benzene, ether, toluene, ethyl acetate, chloroform, acetone, carbon tetrachloride and suitable combinations of these solvents failed to give any separation.

By spreading the paste on a porous plate, the oil was absorbed, and a solid left almost free from oil. This solid was then scraped off, dissolved in hot ligroin or ligroin-benzene mixture, and on cooling crystallized beautifully in rosettes of rhombic plates. Yield, 23 gm., m.p. 109° C. Analysis: calcd. for $C_{12}H_{13}O_4Br$, Br, 26.60; found, 26.54%. Further experiments showed this substance to be 1:3-bromoethylidene glycerol 2-benzoate.

The oil absorbed by the porous plate was extracted with ether in a Soxhlet extraction apparatus, the ether recovered by distillation and the oil thus obtained fractionated under reduced pressure, giving 175 gm. (45.6% yield) boiling at 170° C./0.6 mm. (bath temp. 205° C.); n_D^{25}, 1.5452; d_4^{25}, 1.4618. Analysis: Calcd. for $C_{12}H_{13}O_4Br$, Br, 26.60; found, 26.46%. Further experiments showed this to be 1:2-bromoethylidene glycerol 3-benzoate. Total amount of pure benzoates obtained, 175 gm.+23 gm.=198 gm. Total yield of pure benzoates, 51%.

The proportion of solid benzoate to liquid benzoate is thus 23:175 or 1:8. This proportion is obtained only when the crude bromoethylidene glycerol is treated with HCl prior to formation of benzoates. An experiment carried out as above, but without a pre-treatment of the bromoethylidene glycerol with HCl, gave a proportion of solid to liquid of only 1:15. Thus HCl appears to cause a shift towards the six-membered isomer in the mutual interconversion of five- and six-membered acetals which takes place under the influence of acid (6). The proportions given above are only approximate and vary slightly in different experiments.

Hydrolysis of 1:2-Bromoethylidene Glycerol 3-Benzoate

Fifty-six gm. of 1:2-bromoethylidene glycerol 3-benzoate was treated with 12 gm. of sodium hydroxide in 120 cc. of water together with 10 cc. of 95% alcohol. The mixture was heated to 35° C. and shaken in an automatic shaker for 12 hr., at the end of which time a clear homogeneous solution resulted. Potassium carbonate (100 gm.) was added and the mixture extracted with ether. The precipitated sodium benzoate was filtered off and both the aqueous layer and the sodium benzoate precipitate were extracted several times with ether. The ether solution was dried over anhydrous potassium carbonate and the ether distilled off. The residue was fractionated under reduced pressure, giving 16.9 gm. of 1:2-bromoethylidene glycerol, b.p. 150-151° C./23 mm. Yield, 46%; n_D^{25}, 1.5008; d_4^{25}, 1.6437. Analysis: Calcd. for $C_5H_9O_3Br$, Br, 40.60; found, 40.85%.

Hydrolysis of 1:3-Bromoethylidene Glycerol 2-Benzoate

Thirty-five gm. of 1:3-bromoethylidene glycerol 2-benzoate was treated with 7.5 gm. of sodium hydroxide in 75 cc. of water together with 6 cc. of 95% alcohol, in exactly the same manner as in the preceding experiment. Twenty-four hours were required for complete solution. The six-membered

benzoate seemed to be more resistant to hydrolysis than its five-membered isomer, and also some decomposition and replacement of bromine was noted. After extraction, distillation yielded 3 gm. of 1:3-bromoethylidene glycerol. B.p. 144-145° C./22 mm.; n_D^{25}, 1.5067; d_4^{25}, 1.6557. Analysis: Calcd. for $C_5H_9O_3Br$, Br, 40.60; found 40.47%.

Methylation of 1:2 Bromoethylidene Glycerol

Dimethyl sulphate (25 gm.) and a solution of 15 gm. of sodium hydroxide in 21 cc. of water were allowed to drop from separate tap-funnels into 25 gm. of the well-stirred acetal during a period of two hours, the temperature being kept at 35-40° C. and a slightly alkaline reaction being maintained throughout. A solution of 3 gm. of sodium hydroxide dissolved in 25 cc. water was then added and the reaction mixture heated at 70° C. for one hour. The cooled reaction product was then extracted with chloroform, dried over anhydrous sodium sulphate and distilled; 10.6 gm. of 1:2-bromoethylidene glycerol 3-methyl ether was obtained. B.p. 117.5-119° C./22 mm.; n_D^{25}, 1.4720; d_4^{25}, 1.4694. Analysis: Calcd. for $C_6H_{11}O_3Br$, Br, 37.92; found, 37.95%.

Methylation of 1:3-Bromoethylidene Glycerol

The reaction was carried out in the same manner as with the 1:2-bromoethylidene glycerol, except that the following quantities were used: 1:3-bromoethylidene glycerol, 3.5 gm.; sodium hydroxide in 3 cc. of water, 2.1 gm.; dimethyl sulphate, 3.5 gm. On extracting and distilling 1.1 gm. of 1:3-bromoethylidene glycerol 2-methyl ether was obtained. B.p. 128-129° C./21 mm.; n_D^{25}, 1.4790; d_4^{25}, 1.4973. Analysis: Calcd. for $C_6H_{11}O_3Br$, Br, 37.92; found, 38.15%.

Synthesis of 1:2-Bromoethylidene Glycerol 3-Methyl Ether

Pure glycerol α-methyl ether (82 gm.; b.p. 110° C./13 mm.; n_D^{17}, 1.4462; d_4^{17}, 1.1198), 95 gm. of bromoparaldehyde (crude syrup), and 8 drops of 40% sulphuric acid were heated at 80-90° C. in a round-bottomed flask with vigorous stirring for 24 hr. as in the preparation of bromoethylidene glycerol. No charring occurred, the mixture at the end of this time having a light straw-colored appearance. It was extracted with ether, the ether solution neutralized with dilute sodium hydroxide solution, washed with a solution of sodium bisulphite, then with water and dried over anhydrous sodium sulphate. The ether was distilled off and the residual liquid fractionated under reduced pressure. Sixty-two gm. of 1:2-bromoethylidene glycerol 3-methyl ether (b.p., 117-119° C./23 mm.; n_D^{25}, 1.4708; d_4^{25}, 1.4663) was obtained. Yield, 38%. Analysis: Calcd. for $C_6H_{11}O_3Br$, Br, 37.92; found, 37.80%.

Synthesis of 1:3-Bromoethylidene Glycerol 2-Methyl Ether

Glycerol β-methyl ether (5.7 gm.); b.p. 123° C./13 mm.; d_4^{17}, 1.1306; n_D^{17}, 1.4505 and prepared from 1:3 benzylidene glycerol 2-methyl ether (8), was treated with 6.6 gm. of bromoparaldehyde as in the preceding experiment. There was obtained in the final distillation 3.5 gm. of 1:3-bromoethylidene glycerol 2-methyl ether; b.p., 127-129° C./21 mm.; n_D^{25}, 1.4792; d_4^{25}, 1.4963. Analysis: Calcd. for $C_6H_{11}O_3Br$, Br, 37.92; found, 38.21%.

It is the authors' intention to investigate the action of alkalies on each of the bromoethylidene glycerols.

References

1. Hibbert, H. and Carter, N. M. J. Am. Chem Soc. 50: 3120-3127. 1928.
2. Hibbert, H. and Carter, N. M. J. Am. Chem. Soc. 50: 3376-3388. 1928.
3. Hibbert, H. and Hill, H. S. J. Am. Chem. Soc. 45: 734-751. 1923.
4. Hibbert, H. and Morazain, J. G. Can. J. Research, 2: 35-41. 1930.
5. Hibbert, H., Morazain, J. G. and Paquet, A. Can. J. Research, 2: 131-143. 1930.
6. Hibbert, H., Platt, M. E. and Carter, N. M. J. Am. Chem. Soc. 51: 3641-3644. 1929.
7. Hibbert, H. and Whelen, M. S. J. Am. Chem. Soc. 51: 620-625. 1929.
8. Hibbert, H., Whelen, M. S. and Carter, N. M. J. Am. Chem. Soc. 51: 302-306. 1929.
9. Hill, H. S., Hill, A. C. and Hibbert, H. J. Am. Chem Soc. 50: 2242-2249. 1928.
10. Hill, H. S., Whelen, M. S. and Hibbert, H. J. Am. Chem. Soc. 50: 2235-2242. 1928.
11. Paquet, A. Unpublished research.
12. Stepanow, A., Preobraschensky, N. and Schtschukina, M. Ber. 58: 1718-1723. 1925.

TWO-COMPONENT SYSTEMS INVOLVING COMPOUND FORMATION[1]

By J. Russell[2] and O. Maass[3]

Abstract

The two-component systems ethyl ether-hydrogen chloride and methyl alcohol-hydrogen chloride have been examined in the gaseous state, and from the pressure-volume-temperature relationship of the binary mixture evidence is adduced of the existence of compound formation. The heats of reaction appear to be constant in the temperature range investigated, and are 5400 calories for the ether hydrochloride, and 9200 calories for the alcohol hydrochloride. The pressure-volume-temperature data for ethyl ether, methyl alcohol and ethyl alcohol are given over the temperature range 50-200° C., and over the pressure range below one atmosphere.

When two gases are mixed the pressure-volume-temperature relationship of the mixture is in general different from that calculated on the basis of Dalton's Law. In every case new factors enter which cause aberration from the ideal gas laws.

One such factor is the attraction between the two different species of molecules. When equal volumes of two gases, each at a pressure of one atmosphere, are mixed, a pressure change takes place which is generally very small, but which is dependent on the above-mentioned factor. Occasionally a marked decrease in pressure occurs, far greater than would be accounted for by the attraction indicated in van der Waals' equation, and which is due to a partial combination with the formation of complex molecules. A large number of systems of the former type where no combination takes place have been studied by Sivertz and Maass (5) and by B. P. Sutherland and Maass (6), and the results will be published shortly. In this paper a number of systems of the latter type in which the pressure changes seem to indicate compound formation are described.

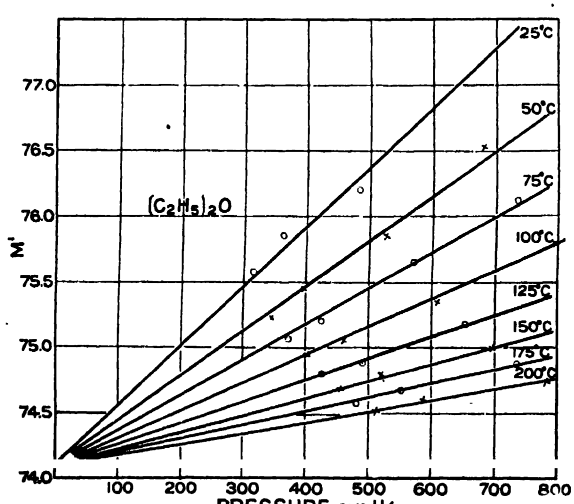


Fig. 1. *Apparent molecular weight of ethyl ether at various pressures and temperatures.*

Alcohols and ethers form molecular complexes (oxonium compounds) with hydrogen chloride when mixed together in the liquid state. Maass and Morrison (3) examined the system methyl ether-hydrogen

[1] *Manuscript received July 16, 1931.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada.
[2] *Holder, at the time, of a fellowship under the National Research Council of Canada.*
[3] *Professor of Physical Chemistry, McGill University.*

chloride in the gaseous state, and showed that an oxonium compound was formed in this case also. This was confirmed independently by J. Shidei (4). The systems described below are ethyl ether-hydrogen chloride and methyl alcohol-hydrogen chloride. Since it was necessary for purposes of calculation to know the pressure-volume-temperature data of the single components these were first determined. Incidentally, these data for ethyl alcohol were also determined.

Preparation of Materials

The hydrogen chloride was prepared by dropping concentrated c.p. sulphuric acid upon concentrated aqueous c.p. hydrochloric acid, the evolved gas being passed through a wash bottle containing concentrated sulphuric acid, then through a tube of phosphorus pentoxide and finally condensed in a tube immersed in a bath of ether cooled to the condensation point of hydrogen chloride by means of liquid air. The liquid obtained was then distilled twice through tubes containing phosphorus pentoxide, the final condensation taking place in a container attached to the density apparatus. It was kept in the liquid state and admitted to the apparatus as desired.

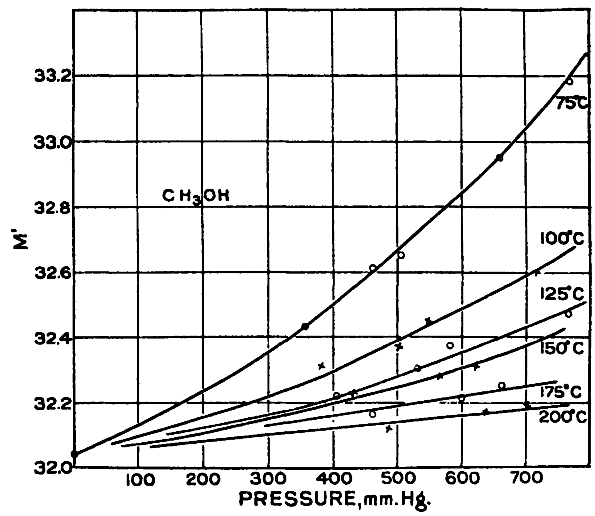


FIG. 2. *Apparent molecular weight of methyl alcohol at various pressures and temperatures.*

The ethyl ether was prepared in the usual manner. Starting with a so-called pure product, it was thoroughly washed with water to remove alcohol and given a preliminary drying by means of calcium chloride. Repeated distillations over sodium gave a product in which no impurity could be detected.

The methyl alcohol was prepared from a c.p. product. It was first treated with a few crystals of iodine to remove the acetone, distilled first over potassium hydroxide and then three times over metallic calcium. The final product showed no sign of impurity.

The ethyl alcohol was treated first with potassium hydroxide to resinify the aldehydes, distilled from the potassium hydroxide and then repeatedly distilled over metallic calcium. (The presence of the aldehyde had not been suspected at the start, so the first preparations were impure.) A careful determination of the density of this final product was made and the result, $d_{4}^{25} = 0.78508$, was found to be in almost exact agreement with the Bureau of Standards value, 0.78506. In point of fact the actual experimental work reveals any appreciable amount of impurity which may be present in any of

the substances, since the extrapolation of the isotherms to zero pressure should give the value $M' = M$, where M is the theoretical molecular weight. This was always found to be so except in the case of the first alcohol samples, mentioned above, which were of course discarded.

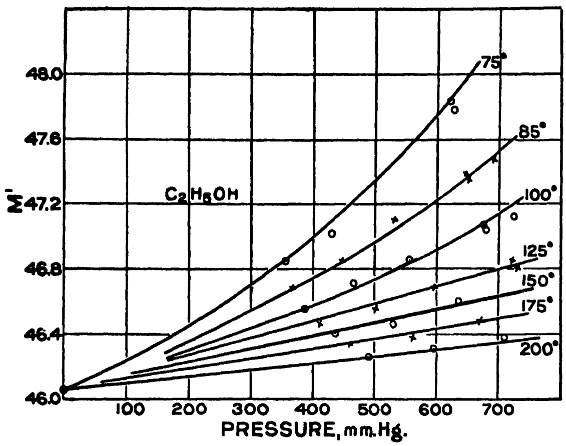


FIG. 3. *Apparent molecular weight of ethyl alcohol at various pressures and temperatures.*

Apparatus and Method

The experimental method was practically identical with that devised by Maass and Mennie (2) for the investigation of substances which are liquid at room temperatures, and for measuring the pressure-volume-temperature relations of a two-component system.

Experimental Results

In Tables I, II and III are given the data obtained for ethyl ether, methyl alcohol and ethyl alcohol. They are tabulated in terms of M', the apparent molecular weight calculated from the quantities observed assuming the ideal gas law to hold.

TABLE I

DATA OBTAINED WITH ETHYL ETHER

Temp., °C.	Mass	Pressure, mm. Hg	Volume, cc.	M'	Temp., °C.	Mass	Pressure, mm. Hg	Volume, cc.	M'
25.0	2.0548	482.5	1039.0	76.203	125.3	2.0523	652.3	1040.1	75.168
25.0	1.5340	361.9	1039.0	75.847	125.3	1.5321	488.9	1040.1	74.870
25.0	1.3355	316.2	1039.0	75.575	125.3	1.3340	426.1	1040.1	74.798
50.1	2.0542	525.2	1039.3	75.862	151.0	2.0519	695.7	1040.3	74.998
50.1	1.5334	394.2	1039.3	75.447	151.0	1.5317	520.8	1040.3	74.786
50.1	2.6796	679.1	1039.3	76.532	151.0	1.3336	454.1	1040.3	74.678
50.1	1.3351	344.2	1039.3	75.232					
					175.5	2.0513	736.6	1040.6	74.882
75.5	2.6787	736.0	1039.5	76.123	175.5	1.5313	551.4	1040.6	74.674
75.5	1.3348	371.9	1039.5	75.070	175.5	1.3333	480.8	1040.6	74.568
75.5	2.0536	567.9	1039.5	75.637					
75.5	1.5329	426.3	1039.5	75.208	201.2	2.0508	779.9	1040.9	74.738
					201.2	1.5309	583.2	1040.9	74.608
100.8	2.0530	611.1	1039.8	75.348	201.2	1.3330	508.4	1040.9	74.520
100.8	1.5325	458.0	1039.8	75.045					
100.8	2.6780	792.6	1039.8	75.780					
100.8	1.3343	399.4	1039.8	74.928					

The discussion of the values for the one-component systems is left in abeyance at present due to the improved method of Cooper and Maass (1) and their modification of the equation proposed by Maass and Mennie (2) which is to

TABLE II

DATA OBTAINED WITH METHYL ALCOHOL

Temp., °C.	Mass	Pressure, mm. Hg	Volume, cc.	M'	Temp., °C.	Mass	Pressure, mm. Hg	Volume, cc.	M'
75.5	1.0373	658.4	1039.5	32.953	151.0	0.7890	621.0	1040.3	32.307
75.5	0.7897	505.8	1039.5	32.656	151.0	0.7184	565.9	1040.3	32.281
75.5	0.7189	461.1	1039.5	32.610	151.0	0.5478	432.2	1040.3	32.229
75.5	1.2210	769.6	1039.5	33.184					
75.5	0.5482	353.5	1039.5	32.436	175.5	0.7888	657.7	1040.6	32.249
					175.5	0.7182	599.6	1040.6	32.209
100.8	1.0371	713.4	1039.8	32.605	175.5	0.5476	457.9	1040.6	32.156
100.8	0.7894	545.6	1039.8	32.451					
100.8	0.7188	498.0	1039.8	32.372	201.2	0.7886	696.3	1040.9	32.189
100.8	0.5481	380.4	1039.8	32.315	201.2	0.7180	634.3	1040.9	32.172
					201.2	0.5475	484.4	1040.9	32.124
125.3	1.0368	762.8	1040.1	32.474					
125.3	0.7892	582.5	1040.1	32.369					
125.3	0.7185	531.5	1040.1	32.298					
125.3	0.5479	406.3	1040.1	32.217					

TABLE III

DATA OBTAINED WITH ETHYL ALCOHOL

Temp., °C.	Mass	Pressure, mm. Hg.	Volume, cc.	M'	Temp., °C.	Mass	Pressure, mm. Hg.	Volume, cc.	M'
75.5	0.9677	430.4	1039.5	47.027	125.3	0.9671	496.4	1040.1	46.545
75.5	0.7972	355.9	1039.5	46.851	125.3	0.7968	409.8	1040.1	46.453
75.5	1.4192	620.5	1039.5	47.838	125.3	1.1618	594.7	1040.1	46.673
75.5	1.4265	624.5	1039.5	47.776	125.3	1.4165	722.4	1040.1	46.845
					125.3	1.4256	727.9	1040.1	46.792
85.6	0.9675	444.4	1039.6	46.851					
85.6	0.7971	367.5	1039.6	46.676	151.0	0.9668	529.2	1040.3	46.454
85.6	1.1623	530.8	1039.6	47.122	151.0	0.7965	436.4	1040.3	46.411
85.6	1.4171	643.7	1039.6	47.376	151.0	1.1615	633.8	1040.3	46.599
85.6	1.5269	692.0	1039.6	47.483					
85.6	1.4262	648.1	1039.6	47.350	175.5	0.9666	560.4	1040.6	46.380
					175.5	0.7963	462.1	1040.6	46.336
100.8	0.9674	464.5	1039.8	46.710	175.5	1.1611	671.7	1040.6	46.480
100.8	0.7970	384.0	1039.8	46.550					
100.8	1.1621	556.2	1039.8	46.861	201.2	0.9663	593.0	1040.9	46.314
100.8	1.4169	675.1	1039.8	47.072	201.2	0.7961	489.1	1040.9	46.261
100.8	1.5267	726.6	1039.8	47.123	201.2	1.1608	711.4	1040.9	46.375
100.8	1.4260	679.9	1039.8	47.039					

be published in the near future. In Fig. 1, 2 and 3, M' is plotted against the pressure. In the cases of methyl and ethyl alcohols the curvature of the isotherms at the lower temperatures indicates a certain amount of association.

The data for the two-component systems are given in Tables IV and V. The pressure calculated is obtained from the data given above, and the previously determined data for hydrogen chloride. The method of calculation is given in the paper by Maass

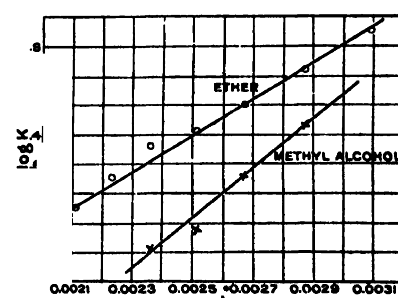


FIG. 4. *Relation between inverse of the absolute temperature and logarithms of the mass law constants for the ether and alcohol systems.*

and Mennie (2). The last column gives the difference in pressure between calculated and observed pressures of the mixtures.

TABLE IV

DATA FOR THE SYSTEM ETHYL ETHER-HYDROGEN CHLORIDE

Temp., °C.	P_{Ether}, mm. Hg	P_{HCl}, mm. Hg	$P_{calc.}$, mm. Hg	$P_{obs.}$, mm. Hg	Difference, mm. Hg
Ether, 1.0019 gm; HCl, 0.5047 gm.					
50.1	258.3	267.1	525.4	512.9	−12.5
75.5	279.0	288.0	567.0	558.8	− 8.2
100.8	299.5	308.8	608.3	602.3	− 6.0
125.3	319.4	328.8	648.2	643.9	− 4.3
151.0	340.2	350.0	690.2	686.3	− 3.9
175.5	360.0	370.0	730.0	727.3	− 2.7
201.2	380.7	391.0	771.7	769.8	− 1.9
Ether, 1.1680 gm.; HCl, 0.5920 gm.					
50.1	300.5	313.3	613.8	596.4	−17.4
75.5	325.3	337.8	663.1	651.6	−11.5
100.8	348.6	362.1	710.7	703.7	− 7.0
125.3	371.9	385.8	757.7	752.0	− 5.7

TABLE V

DATA FOR THE SYSTEM METHYL ALCOHOL-HYDROGEN CHLORIDE

Temp., °C.	P_{CH_3OH}, mm. Hg	P_{HCl}, mm. Hg	$P_{calc.}$, mm. Hg	$P_{obs.}$, mm. Hg	Difference, mm. Hg
75.5	317.2	315.9	633.1	628.5	−4.6
100.8	431.1	338.8	679.9	677.5	−2.4
125.3	363.6	360.9	724.5	723.3	−1.2
151.0	387.4	384.0	771.4	770.4	−1.0

NOTE: *Methyl alcohol, 0.4928 gm.; HCl. 0.5539 gm.*

Discussion of the Results

The deviations tabulated above, *i.e.*, the differences between the observed and calculated pressure, are of a greater magnitude than the changes due to the mixture effect. If this latter is neglected the mass law constant can be calculated on the assumption that the observed changes are due to the existence of the equilibria

$$HCl + (C_2H_5)_2O \rightleftharpoons (C_2H_5)_2O.HCl \text{ and}$$

$$C_2H_5OH + HCl \rightleftharpoons C_2H_5OH.HCl.$$

In Table VI are given the logarithms of the mass law constants for the ether and alcohol systems at various temperatures, and in Fig. 4 these are plotted against the inverse of the absolute temperature.

TABLE VI

LOGARITHMS OF THE MASS LAW CONSTANTS FOR THE ETHER- AND ETHYL ALCOHOL-HYDROGEN CHLORIDE SYSTEMS AT VARIOUS TEMPERATURES

T	$\frac{1}{T}$	log K_{Ether}		log K_{CH_3OH}
		1	2	1
50.1	0.00309	−3.699	−3.682	—
75.5	0.00287	−3.966	−3.950	−4.34
100.8	0.00267	−4.171	−4.239	−4.68
125.3	0.00251	−4.376	−4.388	−5.04
151.0	0.00236	−4.475	—	−5.17
175.5	0.00223	−4.687	—	—
201.2	0.00211	−4.890	—	—

Since the slopes of the curves in Fig. 4 are linear the heats of reaction appear to be constant in the temperature range investigated, and are 5400 calories for the ether hydrochloride, and 9200 calories for the alcohol hydrochloride.

In Table VI it was possible to calculate two values for the mass law constant in the case of the ether from two sets of data in which the amounts of material used were different. The agreement for each pair of values is such as to give additional evidence that the interpretation of the data is based on an assumption which is probably correct.

References

1. COOPER, D. LE B. and MAASS, O. Can. J. Research, 4: 283-298. 1931.
2. MAASS, O. and MENNIE, J. H. Proc. Roy. Soc. Lond. A. 110: 198-232. 1926.
3. MAASS, O. and MORRISON, D. M. TRANS. Roy. Soc. Can. III. 18: 49-55. 1924.
4. SHIDEI, J. Mem. Coll. Sci. Kyoto Imp. Univ. A. 9: 97-119. 1925.
5. SIVERTZ, C., SIVERTZ, V. and MAASS, O. Report to National Research Council of Canada. 1927.
6. SUTHERLAND, B. P. and MAASS, O. Report to National Research Council of Canada. 1929.

THE VAPOR DENSITY OF HYDROGEN SULPHIDE[1]

By R. H. Wright[2] and O. Maass[3]

Abstract

An apparatus for the measurement of the vapor density of condensable gases at pressures greater than atmospheric and at various temperatures is described. The vapor density of redistilled hydrogen sulphide was measured at 47°, 25°, 0°, −20°, and −35° C., and up to 4 atm. pressure. The interpolated value for the vapor density of hydrogen sulphide under standard conditions was found to be 1.537. This value agrees with the values given by Leduc (1.538) and Baume and Perrot (1.539), but the difference may be attributed in part to the neglect of the earlier experimenters to allow for deviations from the gas laws in correcting their values to standard pressure. The results are expressed as apparent molecular weights, and the deviations from the theoretical value shown to be due to the action of molecular attraction.

Introduction

This paper deals with a series of measurements on the vapor density of hydrogen sulphide, undertaken in connection with an investigation of aqueous solutions of the gas and as an extension of the work of this laboratory on deviations from the ideal gas laws. The only precision measurements of this quantity to be found in the literature are those of Leduc (3) and Baume and Perrot (1) which refer only to standard temperature and pressure. Hitherto no comprehensive vapor density measurements above atmospheric pressure have been carried out in this laboratory in checking the modified van der Waals' equation proposed by Maass and Mennie (4), and found to be very satisfactory at the lower pressures. In this work the range has been extended to four atmospheres and the theory found capable of accounting for the facts as observed.

Purification of Hydrogen Sulphide

Compressed liquefied hydrogen sulphide from a cylinder was dried in a trap at −50° C. and purified by low temperature fractional distillation *in vacuo*. The perfect condensability of the product in liquid air and the ability to cross-check vapor density measurements with different batches testified to the purity of the final product.

Measurement of Vapor Density

The procedure was essentially that of Maass and Russell (5) which has also been found to be satisfactory by succeeding workers in this laboratory.

The apparatus is shown in Fig. 1. A calibrated volume, Q, of Pyrex glass, and fitted with a pressure stopcock, P, was connected through a second pressure stopcock, N, with the low pressure portion of the system which included the condensation bulbs, M M, low pressure manometer, F, storage bulb, at G, and pumps. On the high pressure side was the manometer, S, four metres

[1] *Manuscript received August 10, 1931.*

Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada. Constructed from a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry, McGill University.

[2] *Holder of a bursary and studentship under the National Research Council of Canada.*

[3] *Professor of Physical Chemistry, McGill University.*

high. The volume, Q, was of 586.7-cc. capacity at 25° C. and was shaped to fit inside a large Dewar flask used as a low temperature thermostat. This was filled with acetone, stirred by a stream of air and hand regulated to within 0.05° C. For temperatures above 0° C. an automatically regulated water bath was used. Temperatures were read on mercury and alcohol thermometers graduated in tenths of a degree and furnished with calibration certificates by the manufacturer.

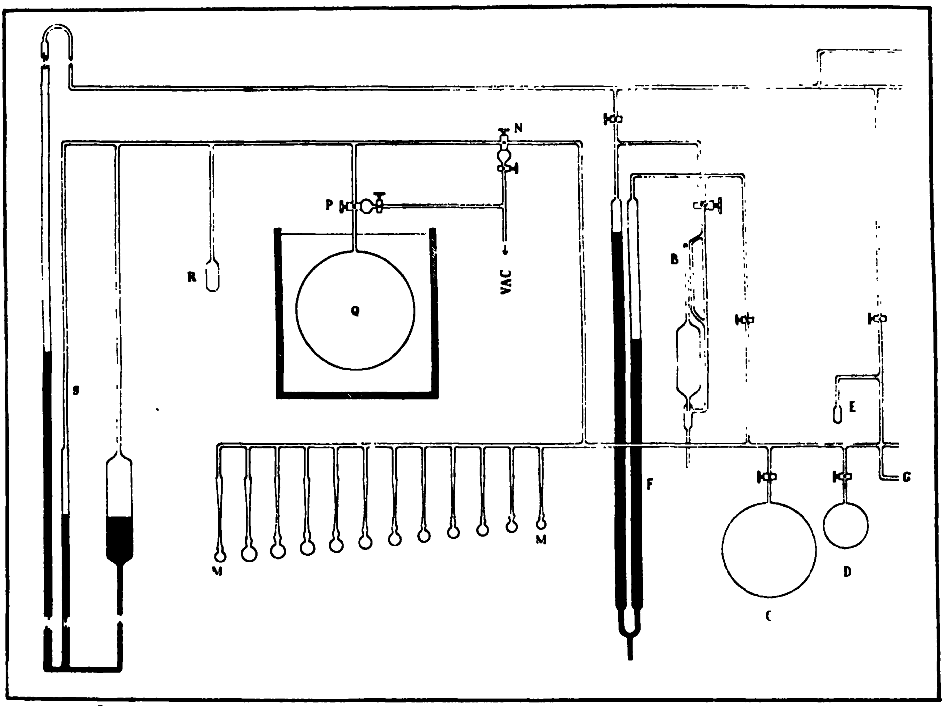


FIG. 1. *Diagram of apparatus.*

In making a determination of vapor density, the whole apparatus was thoroughly evacuated and the gas admitted from G into the entire high and low pressure systems (including C and D) until the pressure was something under an atmosphere. The hydrogen sulphide was condensed in R with liquid air and any residual gas pumped out. Pressure tap N was closed and R allowed to warm up, on which a considerable pressure was developed depending on the original pressure in F. This pressure was measured to the nearest 0.5 mm. on S and tap P closed, whereupon the gas in the tubing was removed. One of the bulbs, M, was then placed in liquid air and the gas in Q condensed into it as completely as possible. Any residual pressure was read on F.

The bulb M was sealed off, allowed to come to room temperature, and weighed to 0.1 mg. It was cooled again in liquid air and the neck broken off (care being taken to lose none of the glass) and the hydrogen sulphide allowed to escape. The empty bulb was dried in a vacuum desiccator and

re-weighed. It was finally filled with distilled water and weighed a third time to determine the volume of air contained in it during the second weighing. The difference between the first and second weighings gave the weight of hydrogen sulphide originally in Q except for the small amount left in the apparatus in sealing off the bulb. Knowing the volume of the tubing and the pressure of the residual gas, this correction was readily found from the ideal gas law, hence the weight of gas initially occupying the known volume, Q, under fixed conditions of temperature and pressure was found. The calculation of vapor density and apparent molecular weight, and the corrections applied to the various measurements were the same as those described by Maass and Mennie (4).

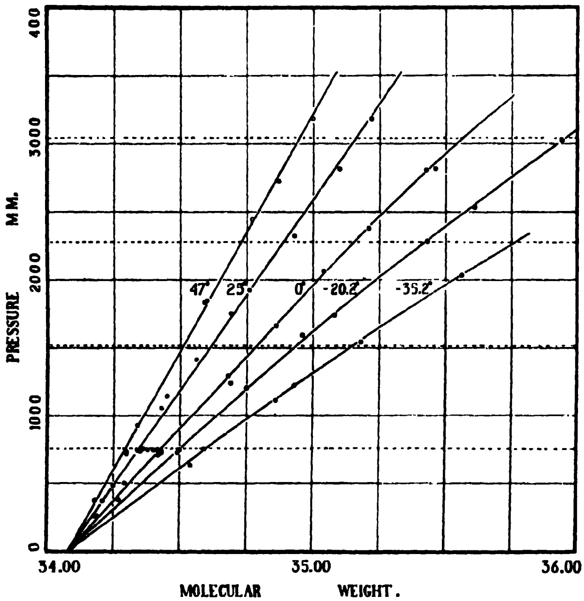


FIG. 2. *Apparent molecular weights of hydrogen sulphide at various temperatures and pressures.*

Experimental Results

The experimental results are tabulated for each temperature in Table I, wherein are shown the pressures (in mm. of mercury at 0° C.) and corresponding apparent molecular weights. These molecular weights are plotted in Fig. 2.

The vapor densities of hydrogen sulphide under stand-

TABLE I
EXPERIMENTAL RESULTS

Temp., °C.	Pressure, mm. Hg.	M'	Temp., °C.	Pressure, mm. Hg.	M'	Temp., ° C.	Pressure, mm. Hg.	M'
47.0	3187	35.00	25.4	750.5	34.36	0.0	740.9	34.42
	2724	34.87	25.1	743.2	34.38		726.2	34.42
	2442	34.77	25.4	743.3	34.34		725.3	34.43
	1850	34.60	25.1	740.3	34.35		382.0	34.27
	1843	34.59	25.4	492.8	34.25	−20.2	3021	35.94
	1148	34.45		502.2	34.29		2537	35.61
	922	34.34	25.5	263.7	34.19		2289	35.43
47.1	735.8	34.30		260.4	34.18		1742	35.08
	718.6	34.30	0.1	2815	35.46		1594	34.96
	375.1	34.21		2814	35.43		1204	34.75
	378.8	34.18		2380	35.21		749.6	34.50
25.0	3185	35.22	0.0	2067	35.04		727.4	34.49
	2819	35.10		1661	34.86	−35.2	2035	35.56
	2321	34.93		1292	34.68		1545	35.18
	1928	34.76		1240	34.69		1228	34.93
	1752	34.69		721.8	34.42		1116	34.86
	1418	34.56		744.7	34.40		751.2	34.59
	1056	34.43		743.1	34.43		631.7	34.54

ard conditions given by Leduc (3) and Baume and Perrot (1) are 1 538 and 1 539 gm. per litre respectively, while the interpolated value for this work is 1.537. This agreement is within the range of experimental error, but the difference may be attributed in part to the neglect of the earlier experimenters to allow for deviations from the gas laws in correcting their values to standard pressure.

Discussion of the Results

The modification of van der Waals' equation proposed by Maass and Mennie (4) is based on the interdependence of van der Waals' b and the molecular mean free path. This, in turn, is known to be influenced by temperature, even at constant volume, due to the action of molecular attraction, as shown by Sutherland. In order, therefore, to obtain an expression giving the variation of b with temperature, the mean free path used in deriving the equation is expressed in terms of the Sutherland viscosity-temperature relation, which may be assumed to define the variation with temperature of the mean free path, corrected for the influence of molecular attraction. The resulting equation takes the form,

$$\left(P+\frac{a}{V^2}\right)\left(V-B\left(1+\frac{c}{T}\right)\right)=RT, \tag{1}$$

or

$$PV^2-RTV+a-RTB\left(1+\frac{c}{T}\right)=0. \tag{2}$$

In the subsequent discussion, the following conventions in symbols will be adhered to: P = pressure in atmospheres, V = volume of 1 gm.-mol. in litres, $R=0.08206$ = gas constant in litre-atmospheres, T = absolute temperature, c = Sutherland's constant, and a and B = constants.

In applying the above equation it is first necessary to evaluate a and B. Knowing the diameter of the molecules and Sutherland's constant for the gas, B can be obtained from the relation,

$$B=\frac{8\sqrt{2}\,\pi r^3 N}{1+\frac{c}{273.1}}, \tag{3}$$

where r = radius of the molecule, N = Avogadro's Number $=6.061\times10^{23}$.

Rankine and Smith (6) give for the viscosity of hydrogen sulphide at 0° C. the value $k=116.6\times10^{-6}$ poise, from which r is obtained by substitution in the following formula, due to Jeans (2),

$$k=\frac{0.499m\bar{C}}{2\pi d^2}, \tag{4}$$

where k = coefficient of viscosity, m = mass of one molecule, $\bar{C}$ = average velocity $=\sqrt{\frac{8}{3\pi}}$ (rt. mean sq. velocity), and d = diameter of one molecule. On making this substitution, $r=2.362\times10^{-8}$ cm.

Returning to Equation 3 and substituting the value for r, then, $B=0.1283$. Writing

$$b=B\left(1+\frac{c}{T}\right), \tag{5}$$

and substituting the Rankine and Smith value for c, *viz.*, 331, the series of

values of b given in Table II are obtained.

TABLE II
VALUES OF b OBTAINED BY SUBSTITUTING RANKINE AND SMITH'S VALUE FOR C IN EQUATION 5

Temp., °C.	47.0	25.0	0.0	−20.2	−35.2
$B(1+\frac{c}{T})$	0.2610	0.2708	0.2839	0.2963	0.3069

In order to evaluate a, the value of b for 0° C. and 1 atm. pressure, and corresponding molecular weight and volume data from the experimental results are substituted in Equation 2, which gives $a = 11.46$.

Equations 1 and 2 can now be applied to the data obtained over the whole range of temperature and pressure. By substituting Equation 5 in Equation 1, at constant temperature, it reduces to $PV^2 - RTV + a - RTb \doteq 0$. Maass and Mennie showed that on substituting $V = \frac{M}{M_0} - \frac{RT}{P}$, the resulting equation could be written,

$$\frac{M}{M_0} = 1 + \left(\frac{a-RTb}{R^2T^2}\right)P + 2\left(\frac{a-RTb}{R^2T^2}\right)^2 P^2 + \text{etc.} \tag{6}$$

Since the series converges rapidly, especially at reasonably high temperatures, they assumed that the apparent molecular weight varies linearly with the pressure, $\frac{M}{M_0} = 1 + AP$, where $A = \frac{a-RTb}{R^2T^2}$.

As will be seen from Fig. 2, this is actually the case with hydrogen sulphide up to quite high pressures, and at temperatures above 0° C. At 0° C., however, a slight curvature can be detected which becomes more pronounced as the temperature falls. It is therefore necessary to take account of the higher powers of P in Equation 6 if high pressures or low temperatures are encountered.

In Table III the molecular weights at 1, 2, 3 and 4 atmospheres pressure, taken from the curves of Fig. 2, are compared with molecular weights cal-

TABLE III
COMPARISON OF CALCULATED AND EXPERIMENTALLY DETERMINED MOLECULAR WEIGHTS OF HYDROGEN SULPHIDE

Temp., °C.	Pressure in atmospheres							
	1		2		3		4	
	Molecular weight							
	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.
47.0	34.30	34.30	34.54	34.52	34.78	34.73	35.02	34.94
25.0	34.35	34.35	34.64	34.62	34.94	34.89	35.26	35.16
0.0	34.43	34.43	34.79	34.79	35.18	35.15	35.58	35.56
−20.2	34.49	34.50	34.96	34.94	35.42	35.42	35.93	35.95
−35.2	34.57	34.60	35.12	35.15	35.67	35.76	—	—

culated from Equation 6 making use of the first two terms of the series in P. The agreement is everywhere within the range of experimental error, with the possible exception of $-35.2°$ C. and 3 atm. pressure. The "observed" molecular weight at this point is taken from the extrapolated curve of Fig. 2, and as this is very close to the condensation point, the point may actually be in the region of supersaturation. Unfortunately the literature contains no data pertaining to the phase equilibria near this temperature.

This is the first time this curvature of the molecular weight versus pressure isotherms has been compared with the curvature predicted by the theory, and the closeness of the agreement is entirely satisfactory.

References

1. BAUME, G. and PERROT, F. L. J. chim. phys. 6: 610-619. 1908.
2. JEANS, J. H. The dynamical theory of gases. 3d. ed. Cambridge Univ. Press. 1921.
3. LEDUC, A. Compt. rend. 125: 571-573. 1897.
4. MAASS, O. and MENNIE, J. H. Proc. Roy. Soc. Lond. A, 110: 198-232. 1926.
5. MAASS, O. and RUSSELL, J. J. Am. Chem. Soc. 40: 1561-1573. 1918.
6. RANKINE, A. O. and SMITH, C. J. Phil. Mag. 42: 615-620. 1921. International Critical Tables, Vol. 5, p. 3. 1929.

A MODIFIED FLOW METHOD FOR MEASURING THE VELOCITIES OF GAS REACTIONS[1]

By E. W. R. Steacie[2] and H. A. Reeve[3]

Abstract

A modified flow method has been devised for measuring the velocities of gas reactions, which enables observations to be made by pressure readings rather than by tedious analytical methods. In principle the method consists of passing the reactants through a flow-meter, then through a heated reaction chamber, and finally through a second flow-meter. The ratio of the two flow-meter readings, corrected for the change in the viscosities of the gases, gives a direct measure of the extent to which the reaction has progressed. As a test of the method the thermal decomposition of ammonia on the surface of silica has been investigated.

Introduction

Two main methods have been used for the kinetic investigation of gas reactions. In one of these the pressure change is measured as the reaction proceeds in a bulb at approximately constant volume. This method possesses the advantages of simplicity and directness of measurement. It has, however, two main disadvantages. In the first place the method is not suitable for the investigation of very rapid reactions. In addition, the analysis of the products of the reaction is difficult, on account of the very small quantity of material formed.

In the flow method, the reactants are passed through a hot tube, and the rate of the reaction is deduced from the rate of flow and the analysis of the products of the reaction. The main advantage of this method is the large amounts of the products which are available for analysis. Its main disadvantage is the large number of analyses which are necessary in order to determine the rate of the reaction.

The present communication deals with an attempt to devise an apparatus which will combine the advantages of the two methods discussed above. As a test of the method, the thermal decomposition of gaseous ammonia has been investigated.

Apparatus

In principle the method consists of passing the gaseous reactants through a flow-meter, then through a hot tube in which partial reaction occurs, and finally through a second flow-meter. If in the reaction a change in the total number of molecules occurs, then the amount of gas passing the second flow-meter will differ from the amount passing the first.

If the viscosity of the mixture of products is the same as that of the mixture of reactants, the difference between the two flow-meter readings will be a direct measure of the extent to which the reaction has progressed during the time that the reactants were in the heated tube. The method thus allows the

[1] *Manuscript received May 30, 1931.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada.
[2] *Assistant Professor of Chemistry, McGill University.*
[3] *Demonstrator in Chemistry, McGill University.*

actual observations to be made by means of pressure readings on the flow-meters, and at the same time provides ample material for analysis.

In general, however, the viscosity of the mixture of gases formed will not be the same as that of the reactants. The difference between the flow-meter readings will therefore not be a direct measure of the extent to which the reaction has progressed. The amount of reaction may however be calculated either from a knowledge of the viscosities of the gases concerned, or from the ratio of the two flow-meter readings when the conditions are such that the reaction goes to completion.

The apparatus is illustrated in Fig. 1. The reaction chamber was a transparent quartz tube, *D*, on which was wound two layers of 16 gauge nichrome wire, separated from the quartz tube and from each other by asbestos paper. On the outside of the windings a further layer of asbestos paper, about one inch thick, was wound for heat-insulating purposes. The windings could be used either in series or in parallel, the current being controlled by suitable rheostats.

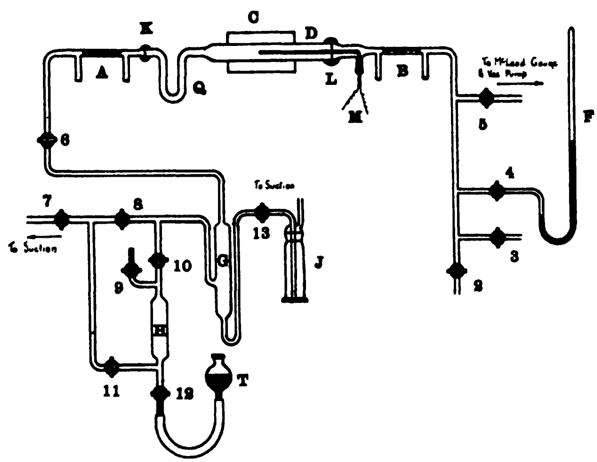


FIG. 1. *Diagram of apparatus.*

At each end of *D* the flow-meters, *A* and *B*, were connected by deKhotinsky seals, *L* and *K*. To prevent softening of the wax, these seals were cooled by a stream of water. The volume of gas (uncorrected for viscosity changes) passing through *A* and *B* was given by pressure readings on the attached manometers, which were filled with paraffin oil. The method of calibration of these flow-meters will be described later. The temperature of the reaction chamber was given by the chromel-alumel thermocouple, *M*, in conjunction with a Leeds and Northrup potentiometer indicator. The temperature could be controlled and read with an accuracy of about 2° C. The thermocouple was enclosed in a thin quartz tube to prevent any possible catalytic action of the chromel or alumel. The leads were taken out through a side tube and deKhotinsky seal, as shown.

The hot gases leaving the reaction chamber were cooled in their passage through the quartz U-tube, *Q*, which was immersed in cold water.

The reactant (ammonia) was introduced from a cylinder, and its rate of flow controlled by tap 3. Its pressure was given by the mercury manometer, *F*. Tap 5 led to the pumping system, which consisted of a Langmuir pump backed up by an oil pump together with a McLeod gauge.

The trap, *G*, was intended for the condensation of liquid products of reaction. These could then be transferred to *J* by suction and withdrawn for analysis.

Gaseous products were drawn into H, and withdrawn for analysis through tap 9. With ammonia, when samples were not being kept for analysis, the unchanged reactant and the products of the reaction were finally passed through tap 7, and then bubbled through water and dilute hydrochloric acid.

Calibration

To determine the quantity of gas passing through the apparatus, corresponding to any given readings of the gauges, the following method of calibration was used. Two Drexel gas washing bottles containing water were connected in series to the apparatus at tap 7. Ammonia was then passed through the apparatus at a constant rate, as indicated by the gauge readings, for a measured time interval. The bottles were weighed before and after, and the volume of ammonia passing through the apparatus per minute was calculated on the assumption that ammonia obeys the ideal gas law at room temperature and atmospheric pressure. The relation between rate of flow and gauge reading was linear within the experimental error.

Since the two flow-meters were not of exactly the same dimensions, the readings on them for the same rate of gas flow differed slightly. They were therefore calibrated one against the other. The relation between the two gauges was also practically linear.

Experimental

The furnace was first brought to the desired temperature. Ammonia was then admitted through tap 3, and its rate of flow adjusted by means of the valve on the ammonia cylinder to give some constant reading of gauge B. If the temperature of the furnace were high enough, some of the ammonia decomposed on passing through C. The reading on gauge A was therefore higher than that of gauge B, since the decomposition of ammonia is attended by a volume increase, and, in addition, the viscosity of the mixture of products is higher than that of ammonia. Readings were taken when gauge A indicated a constant value, showing that equilibrium conditions had been established in C. A series of observations was made in this way with various rates of flow. Experiments were carried out over a temperature range from 900° to 1300° C.

To determine the effect of surface on the velocity of decomposition, another series of runs was made with the reaction chamber packed with small quartz rods.

With the packed tube, it was possible to obtain complete decomposition of the ammonia at measurable rates of flow at the higher temperatures employed. It was found that when the ratio of the two gauge readings, expressed as litres of ammonia per minute, was 2.69, analysis of the products showed that complete decomposition had occurred. This ratio therefore represented the maximum possible decomposition, and further increasing the temperature had no effect.

Since the decomposition of ammonia proceeds according to the equation

$$2\,NH_3 \longrightarrow N_2 + 3\,H_2,$$

we would expect that the volume of gas passing the second flow-meter would be double the volume passing the first when complete decomposition occurs. We would therefore expect a maximum ratio of the two flow-meter readings of 2.0.

The higher ratio actually obtained, *viz.*, 2.69, is presumably due to the fact that the viscosity of the hydrogen-nitrogen mixture formed is not the same as that of ammonia.

The gauge reading of a capillary flow-meter can be represented as follows (1):

$$h = \frac{K_1 \eta R l}{d^4} + \frac{K_2 D R^2}{d^4},$$

where h = gauge reading, K_1 and K_2 = constants, η = viscosity, R = rate of flow, l = length of capillary, d = diameter of capillary and D = density of the gas. The second term in this equation makes allowance for end effects, and can be neglected since long capillaries were used. For the same flow-meter, we may therefore write

$$h \propto \eta R$$

or in other words, the gauge reading will be proportional to the rate of flow and to the viscosity of the gas.

It follows, therefore, that the ratio of gauge readings, corresponding to complete decomposition, should be $\frac{2.0\ \eta_1}{\eta_2}$, where η_1 is the viscosity of a 3 to 1 hydrogen-nitrogen mixture and η_2 that of ammonia.

These viscosities have not been determined experimentally at very high temperatures. According to Kleint (4) the viscosity of a 3 to 1 hydrogen-nitrogen mixture is 0.000134 at 0° C. At this temperature the viscosity of ammonia is 0.000093 (5). The ratio of the two viscosities is 1.44. Hence, if the temperature coefficients of the two viscosities were identical, the limiting ratio of the two flow-meter readings would be 2.88. This is sufficiently close to the observed value of 2.69, since the assumption of identical viscosity-temperature coefficients is certain to be in error.

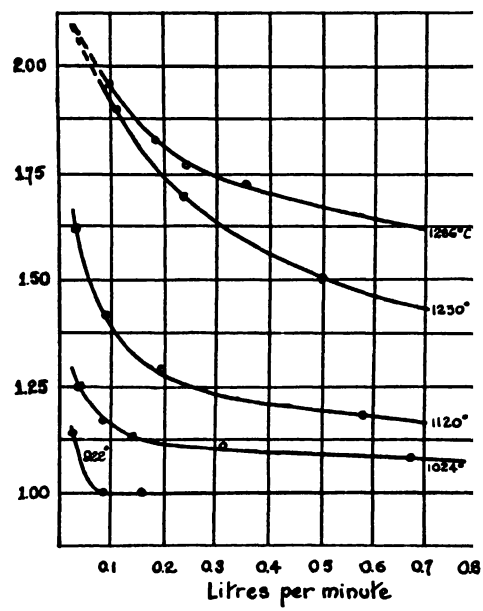


FIG. 2. *Flow-meter ratio—rate of flow curve with empty reaction chamber.*

Since the reaction is heterogeneous, the rate was much slower when the reaction chamber was not packed with silica rods. In consequence, a sufficiently high temperature could not be reached for complete decomposition. The maximum ratio obtainable in this case was about 2.0. Analysis of the products of the reaction, however, showed that some ammonia

was still undecomposed. The amount of ammonia agreed with the assumption that the reaction had only gone $\frac{2.0}{2.69}$ to completion.

The ratio 2.69 may therefore be taken as denoting complete decomposition, and the amount of decomposition under any conditions can be calculated from the ratio of the two flow-meter readings.

Calculations and Experimental Results

The complete data for a typical run at 1120° C. with an unpacked tube are given in Table I.

TABLE I

DATA FOR A TYPICAL RUN AT 1120° C. WITH AN UNPACKED TUBE

Gauge *B*		Gauge *A*		Ratio $\frac{A}{B}$	% Reacted	Time sec.
Mm. of oil	Litres per min. (expressed as NH_3)	Mm. of oil	Litres per min. (expressed as NH_3)			
10.5	0.045	17	0.071	1.57	33.8	25.6
20	0.100	30	0.137	1.37	21.9	12.2
40	0.188	54	0.240	1.27	16.0	6.7
60	0.270	77	0.332	1.23	13.6	4.7
80	0.355	100	0.425	1.19	11.2	3.7

The first and third columns represent the actual experimental observations on the two flow-meters. The second and fourth columns represent these readings converted to give the volume of gas passing each flow-meter in unit time. These volumes are expressed as though the gas in each case were pure ammonia. The ratio of the two flow-meter readings is given in the fifth column. This serves as a measure of the extent to which the reaction has progressed during the time that the reactant was in the hot part of the tube. The values of this ratio at various temperatures and rates of gas flow are given in Fig. 2 for the empty tube and in Fig. 3 for the tube packed with quartz rods. The per cent reaction is given in the sixth column. This is calculated on the assumption that a ratio of 2.69 corresponds to complete decomposition, and that the per cent reaction and the ratio of the flow-meter readings vary in a linear manner.

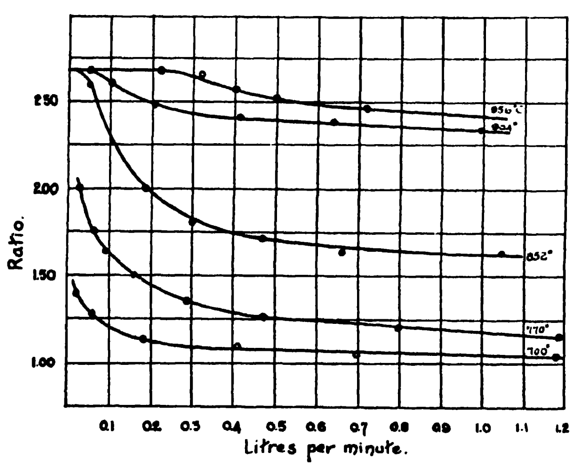


FIG. 3. *Flow-meter ratio—rate of flow curve with reaction chamber packed with quartz rods.*

In order to obtain typical reaction-velocity curves giving the amount reacted

corresponding to various times, it is necessary to know the time during which any amount of gas is in the furnace. This may be determined from a knowledge of the rate of gas flow and the volume of the furnace. Some uncertainty arises, however, on account of the fact that the amount of gas, and hence its rate of flow, changes continuously throughout the furnace as reaction occurs. The actual relationship between the rate of flow and the distance along the furnace is complicated. For our purpose, however, it will be sufficiently accurate to assume that the rate of flow through the furnace is the mean of that corresponding to the initial volume of the gas as it enters the furnace and the final volume as it leaves (these volumes, of course, being calculated at the temperature of the reaction chamber). The times calculated in this way are given in column 7 of Table I. Fig. 4 gives typical per cent reaction-time curves calculated from the data of Fig. 3.

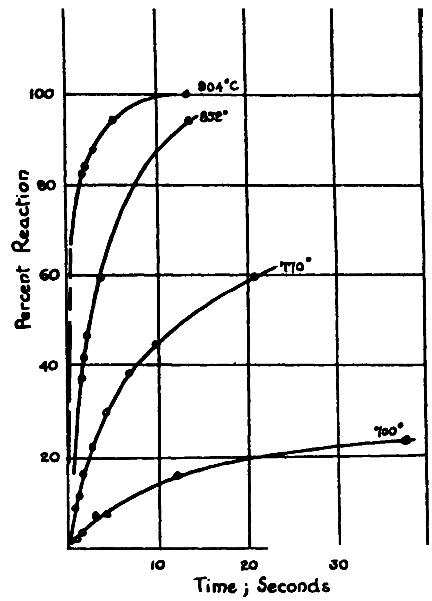


FIG. 4. *Typical per cent reaction—time curves calculated from the data of Fig. 2.*

Discussion

Since the present communication is merely a description of the method, the complete results obtained with ammonia will not be given here except in so far as they have a bearing on the reliability of the method of measurement under discussion.

The results obtained are in agreement with what would be expected from theoretical considerations, *i.e.*, the curves given in Fig. 2 and 3 approach the theoretical straight lines for an infinitely fast and an infinitely slow reaction as the rate of flow and the temperature are varied.

The actual results with ammonia confirm the reliability of the method since they are in general agreement with those of Bodenstein and Kranendieck (2, p. 99) and of Hinshelwood and Burk (3). The reaction has no definite integral order, either the first or the second, since the adsorption of hydrogen formed during the reaction retards the rate. The retardation found was in general agreement with the results of Hinshelwood and Burk.

As far as the method is concerned it may be said that considerably greater accuracy would be needed to make it of general applicability. This could probably be attained by the use of more sensitive manometers of the differential type. The complicated nature of the hydrodynamics of the system is also somewhat of a drawback. Such complications, however, are common to all flow methods.

There are, however, two specific cases in which the present method should prove of value. First, in the investigation of the decomposition of organic compounds, particularly the gaseous hydrocarbons. In such reactions direct readings of the volume change accompanying the reaction could be obtained, together with the possibility of collecting large amounts of liquid products which might be formed in very small amount. Secondly, it should prove useful in giving a continuous direct reading method of determining the yield in industrial catalytic reactions.

References

1. Benton, A. F. J. Ind. Eng. Chem. 11: 623-629. 1919.
2. Bodenstein, M. and Kranendieck, F. Nernst's Festschrift (Halle). 1912.
3. Hinshelwood, C. N. and Burk, R. E. J. Chem. Soc. 127: 1105-1117. 1925.
4. Kleint, F. Verhandl. deut. physik. Ges. 7: 146. 1905.
5. Vogel, H. Ann. Physik. 43: 1235-1272. 1914.

ECONOMY OF TIME IN LABORATORY DISTILLATION[1]

By D. F. Stedman[2]

Abstract

The mathematics of fractional distillation of ideal mixtures has been condensed, so that the most economical "reflux ratio" for any such mixture may be decided at once.

Particular use is made of the "critical reflux ratio" for any mixture, above which even an infinite column cannot obtain complete separation; and the relation of this critical value to the most economical value for any particular case is given.

Some of the conclusions with respect to the infinite column were tested by means of a mixture of methyl and ethyl alcohols using a particularly efficient column. It was found that the vapor produced in the still contained slightly more than the theoretical amount of methyl alcohol, and the magnitude of such error is illustrated from previous work on glycerine solutions.

The results are given in the form of a graph of the "critical reflux ratio" for the case where the most volatile constituent boils at 100° C., and the difference between the boiling points varies from 0.25° C. to 32° C., the concentration of the most volatile constituent also being included from 0.001 to 1.0.

A table of correction factors is also given, showing the factor by which the "critical reflux ratio" should be varied to produce the greatest economy of time for any particular case.

This paper is intended primarily as a condensation of the available data on fractionation of ideal mixtures, in such a form that it may be of direct application to laboratory work. Many chemists may almost be said to spend their lives using distillation as a casual tool for the separation of every conceivable mixture, in much the same manner as others more fortunately situated use filtration.

Distillation is however a much more complex operation than filtration, and in work which has been under way for some time a very serious need has been felt for condensed data which would give some idea of what might be reasonably expected from any particular distillation.

A very large number of distillations are made even on entirely unknown synthetic mixtures, where the only guiding data may be a rough idea of the boiling points of the constituents into which the mixture separates from a preliminary distillation. In such cases where no vapor data can possibly be available, and in others which do not warrant the preliminary study necessary to obtain such data, the use of mathematical calculations will obviously give only the roughest idea of what to expect, but even in these cases some suggestions may be obtained as a guide.

Much data may be obtained from the literature with reference to bubbling columns, but the laboratory column is more generally a packed one, or one closely approximating to it mathematically, and in any case no data have been previously presented in a sufficiently condensed form to be readily available at a moment's notice.

This paper therefore develops the equations covering the operation of a

[1] *Manuscript received July 14, 1931.*
Contribution from the National Research Laboratories, Ottawa.

[2] *Assistant Research Chemist, National Research Laboratories, Ottawa.*

packed column separating an ideal mixture, and presents the results in a form which may be applied directly.

The following terms, similar to those used by Walker, Lewis and McAdams (5, p. 597) may be defined:—

x = mol fraction of lighter constituent, a, in original mixture.

x_p = mol fraction of a in product obtained.

y = mol fraction of a in equilibrium vapor produced from mixture. The still is assumed to provide the column with this vapor.

T and $T+\Delta t$ = the respective boiling points of constituents.

P = mols of liquid taken off as product per minute.

V = mols of vapor entering bottom of column per minute.

α = volatility ratio.

By definition

$$\alpha = \frac{y}{1-y} \cdot \frac{1-x}{x}, \tag{1}$$

$$\therefore \quad y = x\left(\frac{\alpha}{1+x(\alpha-1)}\right). \tag{2}$$

Throughout a packed column the rate of doing work is directly proportional to the amount that the vapor diverges from equilibrium with the liquid with which it is in contact.

This fact is put into a basic equation by Thormann (4, p. 89), but although his method may be worked out plate by plate quite readily, the dependence of the result on the reflux ratio is not mathematically self-contained and must therefore be worked out.

Let N = the maximum number of condensations and re-evaporations which may be obtained from the column, when all vapor is condensed and returned as reflux. Under these conditions the liquid is of the same composition as the vapor at all points, and is therefore "one theoretical plate" out of equilibrium with the vapor, and the rate of doing work is at a maximum. Also let the fraction of this amount of work which may be obtained in practice be k.

When taking off P mols of product per minute the number of theoretical plates to which the column and still is equivalent is therefore,

$$kN+1. \tag{3}$$

As by definition each "plate" multiplies the mol ratio of constituents by α, the mol ratio of constituents in the product is therefore

$$\left(\frac{x}{1-x}\right)\left(\alpha^{kN+1}\right), \tag{4}$$

the original evaporation in the still being equivalent to one plate in any case.

$\therefore$ Mol fraction of a in product

$$= x_p = \frac{x\alpha^{kN+1}}{1+x\left(\alpha^{kN+1}-1\right)}. \tag{5}$$

The liquid returned to the still is also not at equilibrium with the vapor and therefore differs from the liquid in the still. Its composition is evidently:—

$$x\left[\frac{\alpha}{1+x(\alpha-1)}\right]^k. \tag{6}$$

By the same method used in solving bubbling columns, *i.e.*, a balance of material entering and leaving the column, the vapor entering must therefore be equal to the product taken off plus the liquid returned to the still.

$$\therefore \frac{xV\alpha}{1+x(\alpha-1)} = \frac{xP\alpha^{kN+1}}{1+x(\alpha^{kN+1}-1)} + x(V-P)\left(\frac{\alpha}{1+x(\alpha-1)}\right)^{k}, \qquad (7)$$

$$\therefore \frac{P}{V} = \frac{1-\left(\frac{\alpha}{1+x(\alpha-1)}\right)^{k-1}}{\alpha^{kN}-\left(\frac{\alpha}{1+x(\alpha-1)}\right)^{k-1}}. \qquad (8)$$

While this is quite a complex equation which does not lend itself to further simplification, it may be evaluated for any particular case quite readily, preferably by inserting definite values for k; x and α being presumably known.

It will be noticed that the reflux ratio used differs somewhat from that usually employed, in that the product taken off is considered as a fraction of the total vapor produced. This has been found somewhat more convenient, but the more usual forms may be found quite readily; thus the fraction $\frac{V-P}{P}$ represents what is usually called the "reflux ratio".

The further assumption is also made that the molar heats of vaporization of the constituents are equal. This assumption is usually quite justified, but in some cases the difference is appreciable. This simply has the effect of changing the reflux ratio as the vapor ascends the column, but does not invalidate the equations if V is used to represent the mols of vapor passing upwards at any particular point.

Before using the above equations a few further useful relations may be noted for the theoretical case where $N=\infty$, as Equations 1 to 8 are all based on finite values of N.

When $N=\infty$, the product is completely pure for all values of $\frac{P}{V}$ less than a cricical value, while above this value impure product is obtained. The total amount of work then obtained from the column is finite, the efficiency factor, k, therefore zero. The same reasoning used in developing Equation 7 then gives:—

$$Vy = Px_p + x(V-P) \quad . \; . \; . \; . \; . \; . \; . \; . \quad (N=\infty), \qquad (9)$$

$$\text{or} \quad \frac{P}{V} = \frac{y-x}{x_p-x} \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (N=\infty). \qquad (9a)$$

This equation is therefore the same as is found for a bubbling column (5, p. 599).

The maximum value of $\frac{P}{V}$ at which pure material may be obtained is given by substituting $x_p=1$, and the equation is meaningless at lower values of $\frac{P}{V}$.

Experimental Test of Equation 9 with a Very Effective Column

Rearrangement of 9a will show that even with an infinite column if the critical rate is exceeded the product simply consists of the pure product which might have been obtained diluted with *original liquid* up to the actual amount of product taken off.

This is a decidedly drastic conclusion, which so far as the author is aware is not emphasized anywhere in the literature and although it was in no way doubted, it was felt desirable to make an experimental test of the result. As a preliminary, Equation 8 was used to calculate the results available from columns where $N = 5$, 10, 20, 40 plates; and Equation 9a for $N = \infty$, when the original liquid consisted of 10% by weight of methyl alcohol in ethyl alcohol. For this case $\alpha = 1.635$, and $x = 0.1378$, and substitution of values of k from 0.05 to 0.95 gives Fig. 1. It will be seen that with $N = 5$ the highest concentration possible is only 75%, while the 40 plates give a result fairly close to the maximum possible, the purest material available, when $\frac{P}{V} = 0$, containing only 2.5×10^{-10} of ethyl alcohol.

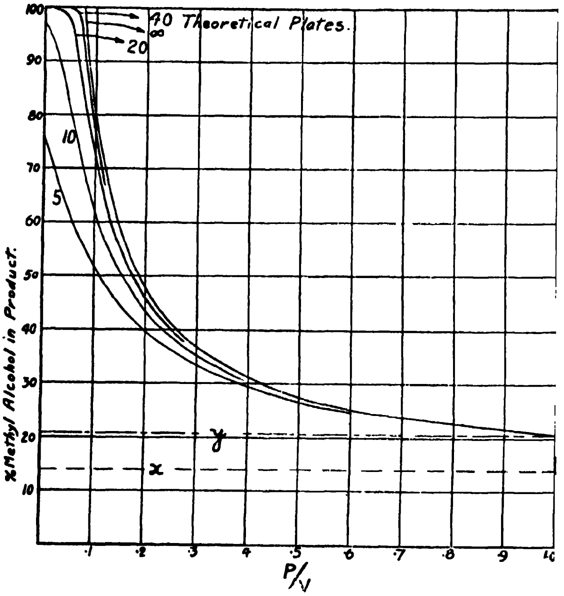


FIG. 1. *Distillation of ethyl alcohol + 10% by weight of methyl alcohol.*

This mixture was then distilled through a column which has been calibrated on the xylenes, pentane and isopentane, etc. This column gives a maximum number of plates in the neighborhood of 300, and for the present purpose should therefore be practically infinite.

The product ratio was set at the desired value and all product returned to the still continuously. The results obtained are given in Fig. 2, with the theoretical curve included.

In general the theoretical curve is followed, but the points immediately after the critical value are decidedly higher than is calculated. This must be due to a slight error in the assumptions at some point. It cannot be caused by any process inside the column, as such a large number of plates is effectively infinite and the results independent of the column. As the ratios were measured accurately the only remaining assumption which might be in error is that the vapor entering the column is the equilibrium vapor.

This is undoubtedly the source of this error, as it is quite a general phenomenon that the vapor evolved from an actively boiling liquid contains too much of the lightest constituent. This "error" is, in fact, the basis of one of the few successful methods so far applied to the separation of isotopes, the partial separation of mercury by distillation from a superheated surface, by Bronsted and Hevesy.

The importance of this fact cannot be overemphasized as practically all the vapor equilibrium data at present available have been determined by direct

boiling of the liquid under examination, and the statement is frequently made that such a process readily gives accurate results (5, p. 585.) This is unfortunately not the case, although the errors are certainly quite small in most of the work reported, and for the original purpose of the work probably negligible. This situation is entirely changed however when such data are used in calculating results of close fractionation.

That such small errors do exist even with systems studied repeatedly, and that their effect is by no means negligible can readily be seen by examination of data reported in (5, p. 600), which is an attempt to make calculation fit experimental results on the fractionation of ethyl alcohol from water. Examination of their Fig. 150 will show that, above the 30th plate, fractionation is actually proceeding more than three times as fast as the calculated curve shows. This is such a serious difference that even allowing for the fact that the experimental data are stated to be far from complete, it is still quite evidently impossible to increase the assumed plate efficiency, etc., enough to correct the error.

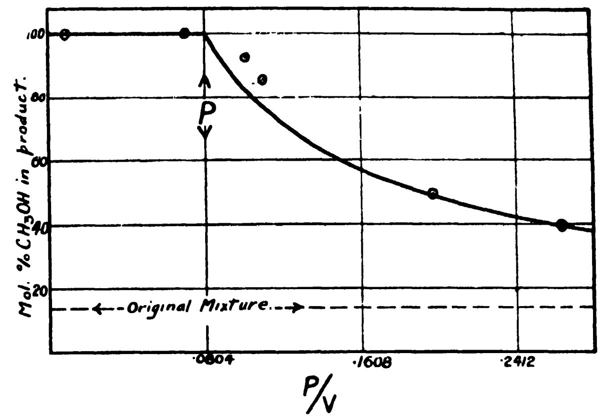


FIG. 2. *Distillation of alcohol mixture through nearly infinite column.*

In this connection reference might also be made to work reported by the author elsewhere (3) on the vapor equilibrium of glycerine solutions. In this investigation a great deal of time was spent investigating precisely this phenomenon, and it was found that gentle boiling was one of the worst methods of obtaining equilibrium vapor. The addition of a platinum "boiling coil" however reduces the error to nearly the minimum, but it still amounts in this case to about 2° C. The method found to give the closest approach to equilibrium was the use of a fairly concentrated source of heat in the form of a "boiling coil" combined with sufficient cooling below the liquid level to prevent too rapid evaporation, the whole apparatus being either thoroughly insulated or placed in a thermostat at exactly the equilibrium boiling point of the solution. With this arrangement it was found possible to reduce the error to slightly greater than 1° C., but the slight gain hardly offset the greater trouble, and the simpler "boiling coil" alone was used, as this error was eliminated further on in the apparatus.

In Table I are given a few unpublished results obtained during the progress of the work reported on glycerine solutions, t being the true boiling point of the glycerine solution at 66 cm. pressure, and Δt being the amount by which the vapor deviated from equilibrium; the solution with an equilibrium boiling

TABLE I

ERRORS RESULTING FROM DIRECT BOILING OF GLYCERINE SOLUTIONS, USING PLATINUM "BOILING COIL"

t, °C.	120	130	140	150	160	170	180	190	200
Δt, °C.	−2.4	−2.0	−4.0	−4.0	−7.5	−5.0	−6.5	−4.0	−4.0

NOTE:—*Abstracted from Ph.D. thesis, University of London, 1924.*

point of 120° C. giving a vapor which would be obtained at 117.6° C. under equilibrium conditions, and similarly with the others. Although this error appears rather small when compared with the difference in boiling points, it corresponds to a vapor containing only 83% of the required amount of glycerine.

In considering mixtures which boil much nearer together, the absolute error naturally decreases, and becomes more difficult to detect, but its relative importance remains approximately constant.

One very unfortunate feature of these small errors is that the application of Duhem's equation will not detect them. As this equation is based on the second law of thermodynamics it is a fundamental property, and even in the presence of serious systematic error, of the type considered, the equation is still satisfied.

The present paper is however not particularly concerned with the actual measurement of vapor equilibrium, beyond emphasizing the extreme degree of absolute precision required in such data before the results are of much value for the purpose of calculating the results of close fractionations, although it might be mentioned in passing that the apparatus suggested by Rosanoff (2) violates the phase rule, as three variables (vapor composition, pressure and temperature) are fixed on a divariant system. Unfortunately the method does not eliminate errors and the original vapor might have been condensed directly.

Possibility of Saving Time in a Fractionation

In starting any particular distillation it is obviously desirable to obtain the result as rapidly as possible. The faster a distillation is performed however the more often it must be repeated, and some particular speed is evidently the most economical.

The work to be done is obviously proportional to the number of mols to be distilled, and may also be considered proportional to the number of theoretical plates, operating at $\frac{P}{V}=0$, which would be required to give the desired separation. The work obtained from any particular distillation is evidently some fraction of this number of plates, and is therefore equal to $kN+1$, and the time spent obtaining one mol of product is proportional to $\frac{V}{P}$.

Combination of these data gives the relative time spent per mol of product, per plate of separation as:—

$$\frac{1}{kN+1}\cdot\frac{V}{P}\,. \qquad (10)$$

However, several factors must still be considered before evaluating such an equation, as repeated distillation has several definite disadvantages.

1. It is never proportionately more effective, some separation being inevitably lost in the process.
2. Losses of material by evaporation, etc., are more serious.
3. More work is entailed in analyzing samples, recording and co-ordinating data, etc.
4. The curves obtained from a redistillation are never as smooth as the first obtained.

All these effects tend to give more weight to the first fraction of (10) than the last, and it does not seem too much to use the $\frac{3}{2}$ power, giving the relative time spent in producing a result as:—

$$\frac{1}{(kN+1)^{\frac{3}{2}}} \cdot \frac{V}{P}\text{, per "plate", per mol.} \tag{11}$$

The $\frac{3}{2}$ power is of course estimated, but the second power is definitely too high.

Using this equation the relative times spent treating the methyl-ethyl alcohol mixture considered in Fig. 1 were calculated for each column, and over the useful range of $\frac{P}{V}$.

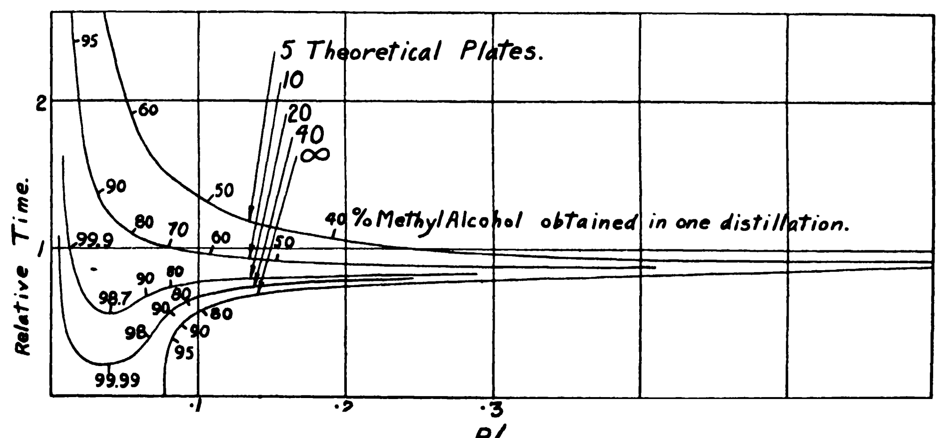


FIG. 3. *Relative times spent distilling alcohol mixture.*

The results of this calculation are given in Fig. 3 in which the times spent per mol distilled, per "theoretical plate" of separation, are plotted against the ratio $\frac{P}{V}$, for each column.

It will be seen that the infinite column theoretically consumes no time at all values of $\frac{P}{V}$ below the critical value (0.08038). This is simply due to the fact that it produces completely pure product at any value of $\frac{P}{V}$ less than the critical, which always represents an infinite number of plates, and the time

consumed per plate separation is therefore zero. It is of interest however to note that if the critical value is exceeded by only 0.00005, raising the ratio to 0.08043, the infinite column is reduced to the equivalent of only 21 theoretical plates. This is an extremely drastic reduction, which is not in any way compensated by the slightly increased rate.

With the 40- and 20-plate columns a sharp maximum of efficiency (minimum of time) is obtained at $\frac{P}{V}=0.04$. This it will be seen is a half of the "critical value".

However, the actual value of $\frac{P}{V}$ used in practice with these columns would be somewhat affected by other considerations, principally the fact that only one distillation is required to give the desired result, and the reflux ratio is therefore set at the *maximum rate* which will still permit sufficiently pure product to be obtained. The purity of the product obtained in one distillation is marked on these curves.

With the 10- and 5-plate columns a still different type of result is obtained, in that the columns are somewhat inadequate, and a decrease of $\frac{P}{V}$ to low values is therefore not compensated by a corresponding gain in purity. These columns however do show a slight minimum time at $\frac{P}{V}=0.4$ and 0.6 respectively, and in cases where the saving of time or money is the principal factor these columns should be used at about those values of $\frac{P}{V}$, but in the laboratory the saving of material and human inertia would combine to suggest that these columns be used at the *lowest value* of $\frac{P}{V}$ which does not introduce serious loss of efficiency. This process combines the maximum amount of separation per distillation with the minimum number of treatments. In the laboratory the "10-plate" column would probably be best used at $\frac{P}{V}=$about 0.08, giving 69% product in one treatment, while the "5-plate" column is probably most economical at about $\frac{P}{V}=$about 0.14, giving 45.5% product the first time.

It will be seen that in the above cases all of the best values of $\frac{P}{V}$ are closely related to the critical value for the infinite column, and in fact only in two cases, when x is very low, and when the column is somewhat inadequate, is it an economy of time to depart very appreciably from this value.

The more general problem may be considered under the following groups:

$x=0.2$ or higher. With an adequate column it is not economical to hurry the process, but to use the highest value of $\frac{P}{V}$ which will give the desired product, usually somewhat less than the "critical value"; while if the column is less efficient a small amount of time may be saved by distilling more rapidly, but the saving is never great.

$x=$less than 0.2. As the concentration of the desired constituent in the original mixture is decreased below 0.2, it becomes more economical to distil

at greater rates, especially if the column is not particularly efficient. The lower x becomes, the lower is the critical value and the greater the economy which may be obtained by distilling at rates greater than the critical. This is the principal case when time may be saved by departing from the critical value. As an approximate guide it is suggested that if $x=0.05$ or less the critical value should be exceeded by the factor $\sqrt{\frac{1}{x}}$.

The difference in boiling point of constituents seems to make very little difference to the above conclusions, although as the boiling points are closer the critical value of $\frac{P}{V}$ naturally decreases (see Equation 9a when $x_p=1$) and the rate of distillation must be correspondingly decreased. The above results are summarized in Table II.

TABLE II

FACTOR BY WHICH CRITICAL VALUE OF $\frac{P}{V}$ FOR INFINITE COLUMN SHOULD BE MULTIPLIED FOR MOST ECONOMICAL WORK

x	Adequate column	Moderately adequate column	Inadequate column
0.2 or higher	0.5 to 1	0.25 to 0.5 or 1 to 1.5	1.5 to 2.5 or more as the column becomes less efficient
0.05 to 0.2	0.5 to 1	0.25 to 0.5 or 1 to 2	2 to 3 or more, as above
Less than 0.05	$\sqrt{\frac{1}{x}}$	$\sqrt{\frac{1}{x}}$	$\sqrt{\frac{1}{x}}$

For the moderately adequate column take the top figure if the column will just give the desired result in one distillation; if two are needed take the lower; while if more than two, consider the column inadequate.

It remains only to evaluate the critical value of $\frac{P}{V}$ for the infinite column for any particular case, to be able to decide at once the most economical value for any laboratory distillation. This calculation has been made for ideal mixtures of constituents which boil from 0.25° to 32° C. apart, and the result plotted on a double logarithmic scale over the range of $x=0.001$ to 1.0, giving Fig. 4.

These values are all calculated for what is considered a "normal" liquid, *i.e.*, one which gives a value of 1.20×10^{-4} in Craft's equation for the correction of boiling points to normal pressure. If the liquids have a lower value than 1.2×10^{-4}, *e.g.*, the alcohols, they are somewhat more readily separated and a proportionately higher rate may be used; while in the reverse case, *e.g.*, chlorinated hydrocarbons, etc., the rate should be correspondingly reduced.

A further assumption was also made that the lighter constituent boils at

100° C. The values of Δt given at the right of the plot are directly proportional to the absolute boiling point of the lighter constituent, and should therefore be correspondingly increased or decreased if it boils materially higher or lower than 100° C.

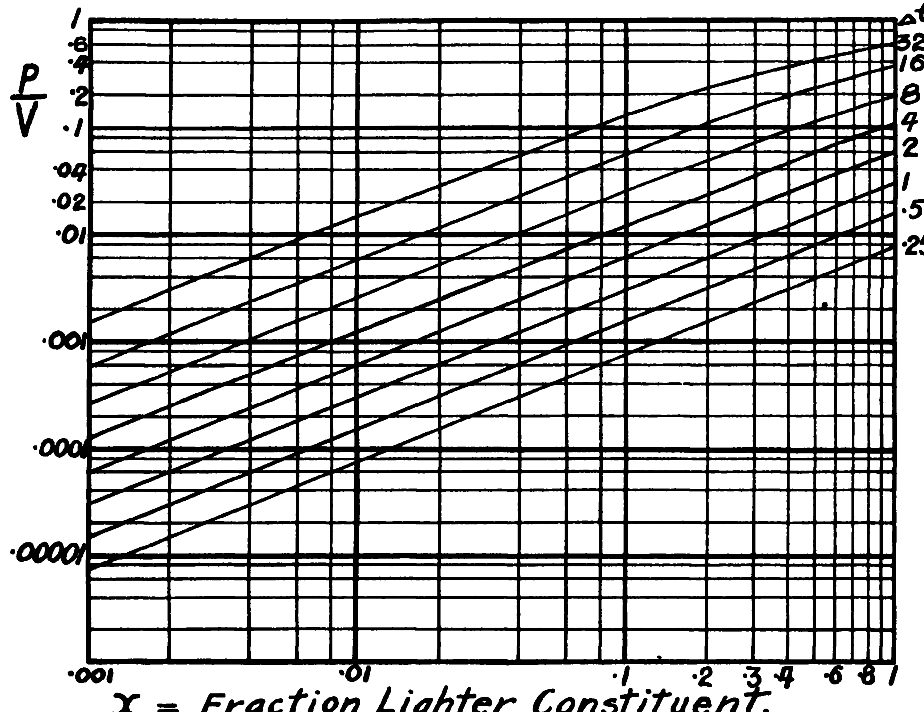


FIG. 4. *Critical values of* $\frac{P}{V}$ *for infinite column.*

A further trouble is, of course, encountered in using these data in the laboratory, in that very few mixtures can be considered ideal. If the deviation is at all serious, accurate calculations are impossible unless the system be studied previously. For a guide in such separations the boiling points of mixtures should be investigated if possible, as the tangent to the boiling point curve, at the concentration considered, extended to the axes, gives a very good estimate of the behavior of the mixture.

When these calculations are made it is rather surprising that the critical values obtained should be so low, *e.g.*, even with a mixture containing 8% of a constituent boiling 32° C. below the remainder, 90% of the vapor distilled into the column must be returned as liquid. This is a much higher reflux ratio than is usually adopted in the laboratory for distilling such a mixture (1, p. 366).

In order to apply the above data to any distillation the following steps are necessary.

1. Calibration of the column, or at least a fairly close idea should be obtained of what separations may be obtained with it.

2. Consideration of the mixture itself, whether it is nearly ideal or otherwise. Known data on compounds chemically similar should be consulted if necessary.

3. The boiling points of the constituents are also required, and at least approximate concentrations.

4. From Fig. 4 the critical value of $\frac{P}{V}$ for the infinite column is obtained for the particular mixture.

5. From Table II suggestions may be obtained giving the amount of departure from the critical value likely to result in the greatest economy.

References

1. Bruum, J. H., Leslie, R. T. and Schicktanz, S. T. Determination of toluene in mid-continent petroleum. Bur. Standards, J. Res. 6: 363-367. 1930.
2. Rosanoff, M. A., Lamb, A. B. and Breithut, F. E. J. Am. Chem. Soc. 31: 448-456. 1909.
3. Stedman, D. F. Trans. Farad. Soc. 24: 289-298. 1928.
4. Thormann, K. Destillieren und Rektifizieren. Verlag von Otto Spamer. Leipzig. 1928.
5. Walker, W. H., Lewis, W. K. and McAdams, W. H. Principles of chemical engineering. McGraw-Hill. 1923.

A SIMPLE APPARATUS FOR PURIFYING RADON[1]

By G. H. Henderson[2]

Abstract

A description is given of a simple and efficient apparatus for purifying radon, which has given several years effective service in connection with radium therapy. Potassium hydroxide is used to remove carbon dioxide and most of the water. Since phosphorus pentoxide for drying has been dispensed with, only one Toepler pump and fewer valves and stopcocks are necessary. The number of stopcocks has been reduced to three and there is but one large bulb and one float valve. The impurities are removed by sparking and allowing excess hydrogen to escape through a palladium tube heated electrically; thus there is no danger of clogging the tap with particles of loose reagent. All parts are made of Gundlach glass. Purification takes from two to five minutes.

In radium therapy, radon is being used more and more widely in place of the radium salt because of its many advantages. When radium is used in this way some form of plant is necessary for pumping off, purifying and tubing the radon. Many descriptions have been published of apparatus designed to fulfil this requirement. While serving their purpose, these plants have generally been very complicated with numerous bulbs, float valves and stopcocks (the latter alone usually numbering eight or more). Highly trained personnel is consequently required to operate the plant. The dangers of breakage and of clogging of valves and stopcocks are ever present in using glass apparatus. These dangers are multiplied in proportion as the apparatus is more complicated.

It seemed feasible to design a greatly simplified apparatus which would give equally good results. This apparatus should be cheaper, less liable to breakage and more easily manipulated than the usual type. These features are very desirable if the use of radon is to be extended so that small centres of population could enjoy its benefits.

Such an apparatus was developed in this laboratory with the aid of a grant from the National Research Council of Canada and a description of it published by W. G. Moran (1, 2). After the experimental plant, set up in the Victoria General Hospital, Halifax, had been in use for three years with very satisfactory results, it became necessary to remove it to another part of the hospital. Advantage of the opportunity was taken to rebuild the plant, incorporating small improvements and paying more attention to appearance. A short description of the plant follows. Many details which have already been sufficiently described in Moran's paper are omitted.

Description of Plant

The method of operation will be clear from a consideration of Fig. 1. The radon is pumped from the radium solution in the flask, 1, by the Toepler pump, 2, into the purifying unit, 3, which had been previously evacuated through the

[1] *Manuscript received August 1, 1931.*
Contribution from the Department of Physics, Dalhousie University, Halifax, N.S.
[2] *Professor of Mathematical Physics, King's College, Dalhousie University.*

stopcock, 6. The impurities are removed by sparking and allowing the excess hydrogen to escape through a palladium tube heated electrically from the outside. A little potassium hydroxide fused to the wall of the unit removes carbon dioxide and most of the water. When purification is complete the radon is simply pushed up into the capillary tube above and sealed off ready for use.

It will be seen that the number of stopcocks has been reduced to three, all of which can be cleaned without admitting air to the solution. There is but one large bulb and one float valve.

It will be obvious to those familiar with the different types of radon plants that this simplification has been obtained by dispensing with the usual phosphorus pentoxide for drying the radon and by the use of palladium. Both of these features are original. The palladium method substitutes a clean physical purification for a chemical one involving frequent renewals of reagents and demanding great care lest the internal heating arrangements burn out. The use of pentoxide has always necessitated a second Toepler pump and numerous valves and stopcocks in order to transfer the purified radon into the capillary. In the present arrangement the radon is pushed straight through the purification unit into the capillary with a minimum of handling. Most of the water impurity is taken up by the potassium hydroxide. The remainder condenses on the walls of the unit as the mercury moves up. A visible layer of condensed water has never been observed on the top of the mercury in the capillary.

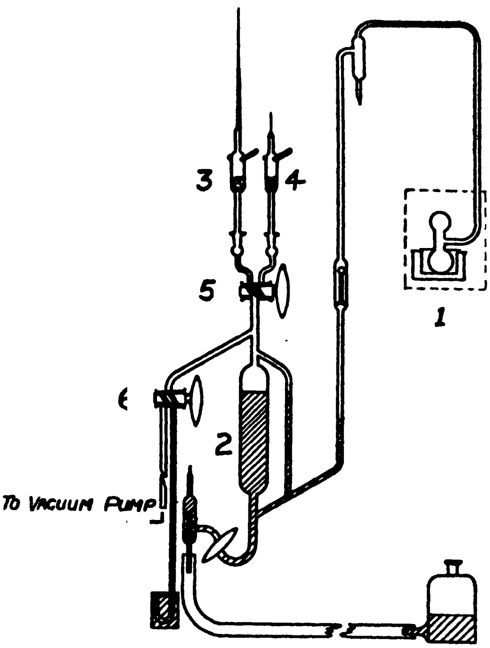


FIG. 1. *Diagram of apparatus for purifying radon.*

An alternative purifying unit, 4, is shown in Fig. 1 which is useful, though not necessary, in preparing gold "seeds". The tube leading from stopcock 6 to the pneumatic trough is not necessary for the process described above, but is used for transferring radon elsewhere if required.

The completed plant is shown in the photograph, Fig. 2. The framework is made of 1¼ in. pipe using stock fittings and angle irons. Provision has been made for a duplicate installation. The radium solution is kept in a commercial safe and is surrounded by over half a ton of lead bricks, giving an average protective thickness of more than five inches. The tube leading to the Toepler pump is protected by lead tubing of ¾ in. wall thickness. The safe and contents are kept cooler than the room by circulating water, thus preventing distillation of the solution into the pump.

The purification unit is evacuated through stopcock 6 by a Hyvac pump. With an occasional cleaning this pump has given a satisfactory vacuum for over three years.

All glass parts of the apparatus are made of Gundlach glass. Attention is drawn to the shape of the flask containing the solution. The upper bulb is for the protection of the solution in case of an inrush of mercury. Against this remote contingency three safeguards have already been provided: (1) a float valve, (2) a trap and (3) by having the tube leading to the solution rise to more than barometric height above the top of the Toepler pump. Should mercury reach the flask in spite of these safeguards the solution would be displaced into the upper bulb of the flask and held safely there. The contingency, as already remarked, is remote but is present in all types of radon plant.

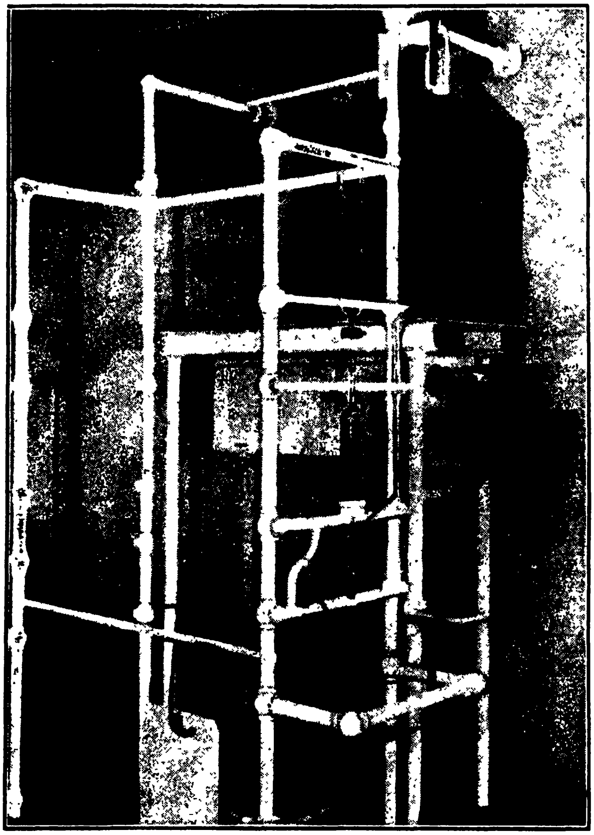


FIG. 2. *Photograph of plant for purifying radon.*

Performance

This type of apparatus has given satisfactory results for over three years. The radon can readily be concentrated to 100 millicuries per cubic millimeter. This would mean, for instance, 10 mc. in a gold "seed" 5 mm. long and 0.15 mm. internal diameter. The apparatus works quickly, the actual purification taking from two to five minutes.

It might be thought that trouble would arise since no particular pains are taken to remove water vapor. In particular there might be fear of occasional blow-outs in sealing off the capillary. No such trouble has developed. Only three blow-outs have occurred since the plant was set up, all caused by attempting to seal off tubes at too high gas pressures. There cannot be more water vapor in the compressed radon than the amount corresponding to the water vapor pressure at room temperature.

The alternative method of purifying by hot copper oxide, described by Moran, has been given up and the palladium tube method used exclusively.

The latter is a purely physical method which is indefinitely reproducible and does not involve any danger of clogging the capillary with particles of loose reagent.

The amount of radium in solution in this apparatus in the Victoria General Hospital is 200 mg. There seems to be no reason why it should not work equally well with larger quantities of radium.

Acknowledgment

The author wishes to thank Mr. S. T. Alvey for the skill and care with which he has constructed the plant.

References

1. Moran, W. G. Phil. Mag. 7: 399-404. 1929.
2. Moran, W. G. Am. J. Roent. August, 1929.

THE ACTION OF HIGH-SPEED CATHODE RAYS ON THE SIMPLER ALCOHOLS, ALDEHYDES AND KETONES, AND ON ETHYLENE[1]

BY J. C. McLENNAN[2], F.R.S. AND W. L. PATRICK[3], PH.D.

Abstract

The results are set forth of an experimental examination of the action of high-speed electrons on the vapors of acetaldehyde, acetone, methyl alcohol and ethyl alcohol, on gaseous formaldehyde and on ethylene. It has been shown that cathode rays form polymerization compounds with formaldehyde, acetaldehyde and acetone and that these suffer decomposition into the simpler gases. The ultimate decomposition of acetaldehyde has been studied in some detail and has been shown to proceed through the polymer. Exposure to the rays results in the decomposition of methyl and ethyl alcohols with the formation of aldehydes, hydrocarbons, oxides of carbon and hydrogen. The conclusion has been drawn that dehydrogenation is the principle primary reaction in the decomposition of the alcohols, further decomposition proceeding through the aldehydes. Ethylene under the action of the rays yields an unsaturated liquid hydrocarbon which upon further bombardment gives hydrogen, acetylene and saturated hydrocarbons.

Introduction

High-speed electrons have been used in studies of the ammonia equilibrium (3, 9), of the polymerization of acetylene (10) and of the oxidation of hydrogen (7), methane and carbon monoxide (8). Other investigations have been concerned with the formation of ozone from oxygen (1), and with the effects of the rays on air, nitric oxide and carbon dioxide (7). The present contribution deals with the changes which occur when the simpler alcohols, aldehydes and ketones, and also ethylene, are subjected in the gaseous state to high-speed electronic bombardment.

Losanitsch (5, 6) performed experiments in the silent electric discharge with the vapors of several substances, including methyl and ethyl alcohols and acetaldehyde. Poma and his collaborators (14, 15) passed the vapors of various alcohols and acetone through an intense discharge from an induction coil and also through a silent discharge. Decomposition was observed in every case, sometimes accompanied by the formation of liquid products. The present investigation has shown that high-speed electrons are capable of decomposing organic compounds; in some cases decomposition proceeds through an intermediate polymeric compound, but with the alcohols dehydrogenation appears to be a favored reaction.

Description of the Apparatus

The cathode ray tube was operated by a transformer system similar to that described by Coolidge (2), Fig. 1. The mains of the 110 volt, 50 cycle current were connected in series with a variable resistance (A), an ammeter (B) and

1 *Manuscript received August 4, 1931.*

Contribution from the Physical Laboratory of the University of Toronto, Canada, with financial assistance from the National Research Council of Canada and the Carnegie Corporation of New York.

2 *Professor of Physics and Director of the Physical Laboratory, University of Toronto.*

3 *Research Assistant, Department of Physics, University of Toronto.*

the primary of a 2:1 transformer (C). The secondary of the transformer supplied current to the primary of a 1:1 insulation transformer (D) having a secondary insulated for 125 kv. The primary of a 200-kv. Snook transformer (E) was fed by the secondary of transformer D. The case of the high tension transformer was insulated from earth and connected to the primary and to the middle of the secondary. One terminal of the secondary was connected to the cathode of the tube and the other was joined to earth through a milliammeter (F). This method of operation was a convenience, since the window of the cathode-ray tube and any apparatus connected to it could be grounded. The window was made of resistal (nichrome steel) foil and was soldered to the brass end-piece.

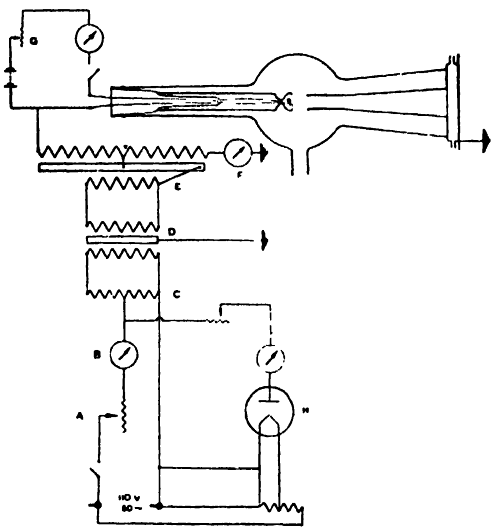


FIG. 1. *The electrical circuit.*

The tungsten filament was heated by a battery of about 12 volts; this battery, an ammeter and the variable resistances (G) were placed in a galvanized iron box, the corners and edges of which were rounded in order to cut down corona discharge. This box was placed on a suitably insulated stand. The current through the filament was controlled by means of two ebonite rods which passed into the box and which were connected to the variable resistances. These were such as to provide a coarse and a finer adjustment.

In order to decrease the voltage of that half of the wave not utilized by the cathode-ray tube and thus to bring the inverse voltage below the "useful", a Tungar valve (H) was connected in parallel across the primary of the transformer C. The voltage applied to the tube was calibrated by means of a standard Victor X-ray spark gap.

The reaction chamber, of a little more than a litre capacity and made of Pyrex glass, was waxed into an annular groove in the brass end-piece, which in turn was sealed by means of wax to the glass of the tube. A stream of water from a thermostat maintained at 20° C. passed through a copper spiral wound round the reaction cell and lagged on the outside, and then through a jacket which served to keep the window at constant temperature and the vacuum-tight wax seals hard. During an experiment, the cathode-ray tube was continuously exhausted through a liquid air trap by means of a mercury-vapor diffusion pump and an auxiliary Hyvac oil pump.

The reaction chamber was connected to a manometer (M), a mercury-filled gas sampling eudiometer, a Sprengel pump (S), and through a side tube to a Hyvac oil pump (P) and to the gas and liquid reservoirs (Fig. 2). The system was constructed throughout of Pyrex glass. Three-way taps permitted of the

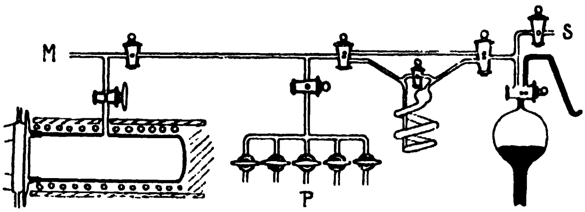


FIG. 2. *The reaction system.*

inclusion of a glass spiral between the reaction chamber and the sampling apparatus; thus the gaseous mixtures could be bubbled through water or passed through a liquid air trap as occasion demanded.

The Initial Substances

Each liquid was obtained in the purest possible form and distilled, an intermediate and constant-boiling distillate being collected in the small vaporizing bulb. This was sealed to the apparatus; subsequently, only vapor at the temperature of the laboratory was allowed to enter the reaction system.

Gaseous formaldehyde was prepared in a similar bulb by heating dried paraformaldehyde. The carbon monoxide and hydrogen used in the experiments with acetaldehyde were prepared in a high state of purity by the recognized methods, washed by suitable reagents, dried by passage over phosphorus pentoxide and stored in two-litre glass reservoirs sealed to the reaction system. Methane was prepared by heating a mixture of anhydrous sodium acetate and barium hydroxide, and purified by passage through glass worms containing fuming sulphuric acid, dilute nitrochromic acid and caustic potash solution. Ethylene was prepared by heating one part of ethyl alcohol with four parts of concentrated sulphuric acid and purified by bubbling through caustic potash solution and concentrated sulphuric acid. Each hydrocarbon was dried by passage over phosphorus pentoxide and liquefied in a liquid air trap. On subsequent distillation, the middle fraction was stored in the aforementioned manner.

Experimental

Prior to any series of experiments the reaction chamber was evacuated; evacuation was continued for several hours with the chamber under bombardment. In this way, the glass was thoroughly outgassed.

The train of tubes leading to the reaction chamber was evacuated and swept out twice with the gas or vapor under investigation. This was repeated with the reaction vessel. Vapor could then be admitted to any desired pressure below that of saturation. The bombardment was commenced and allowed to continue for a definite period, pressure readings being taken at known intervals. The arrangement of the apparatus permitted of the withdrawal of liquefiable gases to a liquid air trap. In this way the partial pressure of any 'permanent gas' formed during an experiment could be measured. Water could be admitted to the trap and later, both gaseous fractions removed for analysis. This permitted of greater ease in the detection of condensable resultants.

After other bombardment experiments with the organic vapors, samples of the resulting gaseous mixtures were withdrawn through 5 cc. of distilled water,

contained in the spiral, and allowed to stand before analysis for several days over mercury in the presence of sticks of zinc chloride. All gas analyses were carried out in a Bone-Newitt gas analysis apparatus, which had been supplemented with a copper oxide tube for fractional combustions. The analyses are recorded on a nitrogen-free basis, adventitious nitrogen being in every case less than 1%. Unless otherwise stated, it may be taken that the values of the applied voltage and tube current during bombardment were 136 kv. and 0.065 milliamperes respectively.

Results

In all, seven series of experiments were carried out, the substances bombarded being gaseous formaldehyde, solid paraformaldehyde, the vapors of acetaldehyde, acetone, methyl alcohol and ethyl alcohol, and finally, ethylene.

Series I and II

Gaseous formaldehyde was allowed to enter the reaction chamber to a pressure of 300 mm. The autopolymerization curve was obtained by plotting pressure against time (curve ABC, Fig. 3). The gas was again admitted, but when the pressure had fallen to 220 mm., bombardment was commenced. The pressure fell rapidly to a minimum value, after which it increased continuously with prolonged exposure to the rays (curve ABDE). The resulting gaseous mixture was freed from aldehyde vapor and analyzed. This procedure was repeated until a point (F) halfway down the sharp fall in pressure was reached, when the bombardment was discontinued and the curve of autopolymerization plotted (curve ABFG). During the course of these experiments, a white solid was deposited on the walls of the reaction chamber. This had been formed by the combined actions of autopolymerization and bombardment. The apparatus was evacuated and the solid subjected to the action of the rays (curve MN) for the same period (250 min.) as in the first and prolonged bombardment experiment, when the resulting gaseous mixture was withdrawn for analysis. The gas analyses of these series are recorded in Table I. No traces of unsaturated hydrocarbons could be detected in these mixtures. The solid removed from the reaction chamber at the end of these experiments was free from carbon.

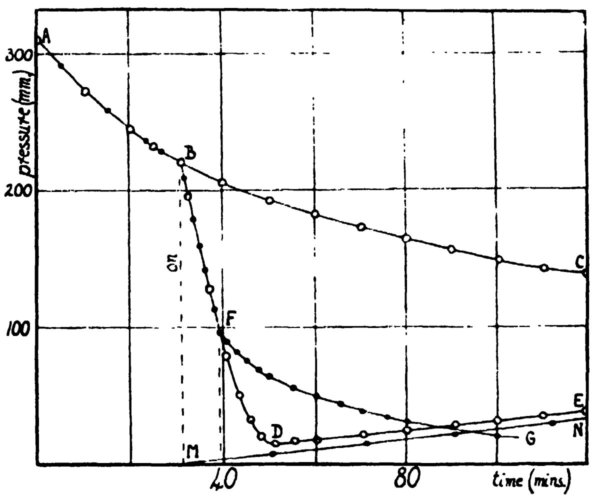


FIG. 3. The curves of Series I and II.

Series III and IV

In these experiments gaseous acetaldehyde and acetone were bombarded at various pressures for known time intervals, the products being withdrawn for

TABLE I

COMPOSITIONS OF THE GASEOUS MIXTURES OBTAINED FROM EXPERIMENTS WITH FORMALDEHYDE

Initial state	Final pressure in mm.	Gaseous mixture			
		CO_2 %	CO %	H_2 %	CH_4 %
Gaseous formaldehyde	69.0	23.6	29.4	39.6	7.4
Paraformaldehyde	68.0	28.0	25.3	40.0	6.7

TABLE II

PERCENTAGE COMPOSITIONS OF THE GASEOUS MIXTURES FORMED DURING THE DECOMPOSITIONS OF ACETALDEHYDE AND ACETONE

Substance	Acetaldehyde		Acetone	
Initial pressure in mm.	190	40	105	105
Time of exposure in hours	4	4	2	4
CO_2	4.2	5.5	7.4	8.1
C_2H_2	1.9	1.3	1.3	1.1
C_2H_4	1.3	1.0	1.2	1.2
CO	37.8	37.2	30.1	29.9
H_2	37.6	39.3	21.5	25.3
CH_4	14.7	13.2	18.0	15.3
C_2H_6	2.5	2.5	20.5	19.1
C/A range (saturated hydrocarbons)	1.79–1.86		1.41–1.49	

analysis. The results are incorporated in Table II, in which a comparison is made of the values of C/A (the ratio of the contraction to the amount of carbon dioxide formed on explosion with excess of oxygen) obtained for the saturated hydrocarbons after the removal of hydrogen, oxides of carbon and unsaturated hydrocarbons. The ranges given include the values from several analyses, usually five or six. From these values, the saturated hydrocarbons were calculated as methane and ethane.

Whereas the bombardment of acetone was always accompanied by an increase in pressure, the sign of the pressure effect with acetaldehyde was influenced by the magnitude of the initial pressure. In an experiment with the initial pressure of acetaldehyde at 190 mm. the rate of change of pressure was negative, indicating that the bombardment was inducing some polymeric change. A yellowish liquid was found to collect within the reaction cell. Eventually after three hours bombardment the pressure reached a minimum value, increasing slightly with further exposure to the rays. On circulating the final gas through the liquid air trap, a residual gas was obtained containing carbon monoxide and hydrogen. The experiment was repeated in order that a complete analysis of the gaseous products might be made. From such data, it was possible to deduce that at pressures in the neighborhood of 100 mm. little change in pressure would be caused by bombardment. In subsequent experi-

ments it was found that no appreciable diminution in pressure occurred with acetaldehyde at an initial pressure of 90 mm.; below this value, however, the pressure was increased.

At this stage the question of the kinetics of these reactions became of importance. Experiments were conducted with acetaldehyde to determine the various influences of (a) the initial pressure, (b) the decomposition products and (c) the applied voltage on the rate of change of pressure at low initial pressures. The pressure-time curves obtained in these experiments were straight lines. The results are recorded in Tables III, IV and V. At voltages below 80 kv. no change could be detected in the contents of the reaction vessel. This, then, was the approximate voltage to be applied to the tube before any electrons passed through the resistal metal window. Above this value, increase in applied voltage resulted in an increase in the rate of decomposition.

TABLE III

INFLUENCE OF INITIAL PRESSURE ON THE RATE OF CHANGE OF PRESSURE OF ACETALDEHYDE DURING BOMBARDMENT

Initial pressure in mm.	4.9	10.8	21.9	24.4	32.5	42.8
dp/dt, mm. per min.	0.020	0.018	0.018	0.018	0.019	0.020

TABLE IV

INFLUENCE OF THE DECOMPOSITION PRODUCTS ON THE RATE OF CHANGE OF PRESSURE OF ACETALDEHYDE DURING BOMBARDMENT

Partial pressure of acetaldehyde in mm.	40.0	40.9	39.5	39.5
Diluent	—	H_2	CO	CH_4
Percentage diluent	—	21.4	19.7	20.6
dp/dt, mm. per min.	0.020	0.022	0.017	0.022

TABLE V

INFLUENCE OF APPLIED VOLTAGE ON THE RATE OF CHANGE OF PRESSURE OF ACETALDEHYDE DURING BOMBARDMENT, THE INITIAL PRESSURE BEING 40 MM.

Applied voltage in kv.	80	90	113	136	160
dp/dt, mm. per min.	0	0.006	0.016	0.020	0.025

After these experiments with acetaldehyde, and those of Series IV with acetone, the respective condensates were removed for examination. In view of the small quantities obtained, this was effected by means of a glass rod. The viscosities increased on standing in air. This effect was more marked in the case of the condensate from acetaldehyde than in that from acetone; otherwise no distinction could be made between the two liquids. They were miscible in chloroform and the solutions thus prepared did not decolorize

bromine. On extraction with water, they yielded a white wax-like solid. It would appear that the condensates are mixtures of this solid and the initial substances, and that the solid is a saturated polymer of a substance capable of being present from either acetaldehyde or acetone, that is, a polymer of formaldehyde or of acetaldehyde. The pressure effects during these experiments are in favor of the latter.

Series V and VI

The vapors of methyl and ethyl alcohols were subjected to bombardment. The pressure increased in every experiment. Neither liquid nor solid products could be detected in the reaction chamber after the experiments with methyl alcohol, but with ethyl alcohol there were slight traces of the presence of a yellow oil. The amount was, however, too minute for removal. The analyses of the gaseous decomposition products are recorded in Table VI.

The aqueous extracts of the resultant gaseous mixtures after experiments in Series IV, V and VI were removed from the spiral and tested for aldehydes. Formaldehyde was present in every case. Quantitative estimations were conducted for the relative amounts of formaldehyde and acetaldehyde present during the decompositions of methyl and ethyl alcohols, the initial pressures being 50 mm. and 28 mm. respectively. Romijn's cyanide method was used for the determination of formaldehyde and Ripper's bisulphite method for total aldehydes. Acetaldehyde was then found by difference. These methods have an accuracy of the order 1 in 100,000 and are uninfluenced by the

TABLE VI

PERCENTAGE COMPOSITIONS OF THE GASEOUS MIXTURES FORMED DURING THE DECOMPOSITIONS OF METHYL AND ETHYL ALCOHOLS

Alcohol	Methyl		Ethyl	
Initial pressure in mm.	51.3	48.4	27.8	28.0
Time of exposure in hours	2	4	2	4
CO_2	9.8	10.9	10.6	10.0
C_2H_2	0.2	0.0	1.4	0.7
C_2H_4	0.6	0.6	2.4	2.1
CO	21.2	22.0	18.5	19.1
H_2	57.0	56.2	47.5	47.8
CH_4	11.2	10.3	16.6	17.1
C_2H_6	—	—	3.0	3.2
C/A range (saturated hydrocarbons)	1.93−1.97		1.7−1.8	

presence of the alcohols in dilute solution. The results are incorporated in Table VII. For purposes of comparison it is convenient to express these results in terms of the original α-carbon atoms recovered as aldehydic carbon. No appreciable difference could be distinguished between the contents of the solutions obtained with the same alcohol after two or four hours exposure to the rays.

Series VII

Bombardment of ethylene results in a fall in pressure with the formation of a liquid product. The rate of change of pressure increases with the initial pressure (see Table VIII) but the relation is not a linear one; the reaction order is greater than unity, indicating that two or more molecules of ethylene are involved. In any experiment the rate of change of pressure falls off more rapidly than would be expected from the change in total pressure. It may be supposed from this that other gases are being formed during the bombardment. The detection of acetylene and of a 'permanent' gas verified this supposition. In later experiments the liquid condensate was bombarded; a gas of the composition $C_2H_2 = 10.7$, $C_2H_4 = 4.5$ and $H_2 + CH_4 + C_2H_6 = 84.8\%$ was liberated, the mixture of hydrogen and saturated hydrocarbons possessing the C/A value of 3.3. The corresponding mixture obtained by bombarding ethylene returned values for this ratio in the neighborhood of 2.2, indicating a lower hydrogen content together with the possibility of the hydrogenation of the ethylene. The analyses were, however, not carried further.

TABLE VII

AMOUNTS OF ALDEHYDES FORMED DURING THE BOMBARDMENT OF METHYL AND ETHYL ALCOHOLS

Alcohol	Time in hours	Weight —CHO in mg. recovered as		Per cent original α-carbon recovered as aldehydic carbon in	
		H.CHO	CH_3.CHO	H.CHO	CH_3.CHO
Methyl	2	2.0	1.8	2.5	2.25
	4	2.0	1.9	2.5	2.4
Ethyl	2	0.6	1.2	1.35	2.7
	4	0.6	1.2	1.35	2.7

TABLE VIII

INFLUENCE OF INITIAL PRESSURE ON THE RATE OF FALL IN PRESSURE OF ETHYLENE DURING BOMBARDMENT

Initial pressure in mm.	84	140	260	400
dp/dt, mm. per min.	12	28	56	128

Mund and Koch (11, 12, 13) and more recently, Lind, Bardwell and Perry (4), have described a colorless liquid obtained by the action of α-particles on ethylene, likening the odor to that of petroleum or of turpentine. The oil obtained in these experiments with high-speed electrons was discolored by carbon and burnt with a smoky luminous flame; it had a high viscosity and a low vapor pressure. The strong odor was similar to that characterizing the higher unsaturated hydrocarbons. The condensate was insoluble in water but readily and completely soluble in chloroform. An apparent partial solubility

in alcohol suggested the presence of two or more components. The color change attendant upon the addition of potassium permanganate in alcoholic solution and also of bromine in chloroform, gave strong evidence of unsaturation.

Discussion of the Results

The pressure-time curves set forth in Fig. 3 enable the conclusion to be drawn that the outstanding action of cathode rays on gaseous formaldehyde is one of polymerization. Bombardment produces a sudden fall in pressure. Cessation of bombardment (as at F) is accompanied by a rapid falling-off in the rate of pressure change. These facts give rise to the view that the rays promote the formation of molecular clusters, which separate from the gas phase as a white solid on the walls of the reaction vessel. Prolonged bombardment resulted in the formation of a residual gas, which subsequent experiments showed to be due to the decomposition of the white polymer. Curve MN almost coincides with curve DE in later stages. The results of the gas analyses given in Table I show that the decomposition is not the simple process which might be expected from single molecules of formaldehyde, but is a more intricate one, involving the breaking up of molecular complexes to yield simple gaseous products.

The straight-line pressure-time curves, the independence of the speed of decomposition of initial pressure (Table III) and the negligible effects of large initial partial pressures of the resultants (Table IV) together establish the fact that the decomposition of gaseous acetaldehyde at initial pressures below 40 mm. is a reaction of zero order unretarded by the resultant products. It may be concluded that within this experimental range and for this value of the applied voltage (136 kv.), at least, the decomposition reaction occurs on the walls of the reaction vessel.

The results given in Table V show that the rate of change in pressure increases with increase in applied voltage; when the latter exceeds 100 kv., however, the rate of change increases less rapidly. This appears to indicate that at voltages above 100 kv., when the gaseous pressure is 40 mm., by far the greater number of electrons are moving with velocities sufficiently large to enable them to reach the walls of the vessel before suffering collisions which are effective in producing molecular disruption. Below this value, the rate of change in pressure is proportional to increase in voltage since the number of effective collisions in unit time in the gaseous enclosure will be proportional to the speed of the electrons and above it because the proportion of the electrons, in a beam of varying velocities, reaching the walls will increase slightly with increase in the energy of the beam. These facts support the conclusion given in the previous paragraph.

The experiments of Series III and IV show that both acetaldehyde and acetone are capable of giving condensates when bombarded and that this effect is more predominant with the former. The analyses incorporated in Table II show that the decomposition products are independent of the pressure and that they cannot be explained by a simple equation. These facts, to-

gether with those established in the kinetic experiments, give rise to the view that the rays promote the formation of clusters as in the case of formaldehyde, these subsequently undergoing decomposition on the walls of the vessel into the simpler gases. The carbon-hydrogen-oxygen balances for these analyses indicate the separation of carbon; it is to this, no doubt, that the condensates owe their color. Precise information as to the part played by the solid constituent of the condensate, which by analogy with the case of formaldehyde is the actual intermediate compound in the decomposition process, was not forthcoming, since sufficient of the solid was not available to permit of purification and subsequent bombardment. It was found, however, that on the window, where it was subjected to the more intense action of the rays, the condensate was highly viscous and contained a large amount of carbon, showing that decomposition of the polymer does indeed occur.

The effect of replacing one or both of the hydrogen atoms in the formaldehyde molecule with methyl groups may be observed in the analyses resulting from acetaldehyde and acetone given in Table II. The introduction of the second carbon atom has resulted in the appearance of small quantities of unsaturated hydrocarbons. The ratio of CO to CO_2 is increased. The ethane present in aldehyde mixtures is only a little above that which might be expected from the action of the rays on the methane present (8). On the other hand, the mixtures resulting from acetone have a high ethane content. This shows not only that two methyl groups on the same carbon atom can combine with the formation of ethane but also that the larger proportion of the methane formed in the acetaldehyde experiments results from the combination of the aldehydic hydrogen and the methyl group. The conclusion may also be drawn that the methyl groups are more remote in the intermediate complex formed during the decomposition of the aldehyde than in acetone; this is supported by the structures of the various polymers of acetaldehyde. But whether acetone first loses $=CH_2$ groups, thereby being capable of forming the same intermediate compound as acetaldehyde with the subsequent combination and hydrogenation of these groups to ethane, or undergoes decomposition by two simultaneous processes cannot at present be settled.

A comparison is made in Table VI of the compositions of the gaseous mixtures formed during the decompositions of methyl and ethyl alcohols. The outstanding point of these analyses is the high proportion of hydrogen. The results of Table VII establish the fact that after an initial change no appreciable accumulation of either formaldehyde or acetaldehyde occurs within the reaction chamber during bombardment. Consequently these substances must be continually decomposing with the formation of further products. Since the experiments of Series II and III show that formaldehyde decomposes more rapidly than acetaldehyde, a greater proportion of the decomposition must occur through the former than through the latter in the case of methyl alcohol. The formation of acetaldehyde during this bombardment must be due to the intermediate condensation of two molecules of the alcohol. With ethyl alcohol the tendency to decompose through formaldehyde is less; consequently, there

is the greater probability of the separation of the acetaldehyde polymer, a fact experimentally observed.

An important feature throughout all these experiments is the low unsaturated hydrocarbon content of the final gases. Indeed, the amount is barely measurable in the cases where the initial molecule contains less than two carbon atoms. As dehydrogenation reactions are promoted with the alcohols, the formation of ketene from acetaldehyde or acetone might be suspected. No trace of acetic acid could, however, be detected after passing the residual gases from the experiments in Series III and IV through water. The absence of higher unsaturated hydrocarbons eliminates any possible explanation of the high carbon dioxide contents by a reaction between ketene and acetaldehyde. Moreover, the low ethylene contents suggest that no ketene is formed since it is unlikely that it would be more stable to cathode rays than were the other substances examined. On the other hand, this almost complete absence of unsaturated hydrocarbons may be due to the fact that they are capable of reacting under the influence of high-speed electrons with the other substances present. But if this were so, then in view of the results obtained with acetone, higher ethane contents would be expected, particularly in the case of ethyl alcohol.

On account of the above facts, the experiments were extended in Series VII to an investigation of the behavior of ethylene under the action of high-speed electrons. Methane reacts only very slowly under the influence of cathode rays, giving small percentages of hydrogen and ethane (8); acetylene is polymerized with the formation of negligible quantities of hydrogen, the solid polymer being unchanged beneath the action of the rays (10). The reaction of ethylene stands between those of methane and acetylene since the oil formed by polymerization, or perhaps condensation, is decomposed on further bombardment into hydrogen, saturated hydrocarbons and acetylene. The possibility of the hydrogenation of the ethylene was not fully exploited. The facts established in the experiments of this series, together with the almost complete absence of unsaturated hydrocarbons in those of Series V and VI, indicate that, in the primary mechanism of the decomposition of the alcohols, a dehydration process can play only a minor part in comparison with one of dehydrogenation.

Summary

(i) Cathode rays rapidly polymerize gaseous formaldehyde and also decompose the resulting solid paraformaldehyde into hydrogen, methane and oxides of carbon. The latter mechanism is an intricate one, involving the breaking up of molecular complexes to yield simple gaseous products.

(ii) Yellow condensates are produced on exposing acetaldehyde and acetone to the action of the rays. These condensates are considered to be solutions of a white polymer of acetaldehyde in liquids which consist almost entirely of the original substance.

(iii) Gaseous mixtures are obtained after bombarding acetaldehyde and acetone. A study of the kinetics of the ultimate decomposition of acetaldehyde

at pressures below 40 mm. shows the reaction to be of zero order unretarded by the resultant products. It is concluded that these gases result from the decomposition of the polymerized aldehyde.

(iv) Cathode rays decompose methyl and ethyl alcohols with but the slightest separation of liquid products in the latter case and none in the former. The amounts of aldehydes present during the bombardment were determined and it has been suggested that the decomposition process consists of dehydrogenation with the subsequent decomposition of the aldehydes.

(v) A yellow condensate is produced on bombarding ethylene with high-speed electrons. This resembles an unsaturated hydrocarbon in its properties and yields hydrogen, acetylene and saturated hydrocarbons on further bombardment.

(vi) It would seem that, in general, the above decompositions occur as condensations. Molecular clusters are first formed under the influence of the rays, and these subsequently set free the simpler gases in an effort to produce more stable configurations.

References

1. Busse, W. F. and Daniels, F. J. Am. Chem. Soc. 50: 3271-3286. 1928.
2. Coolidge, W. D. J. Franklin Inst. 202: 693-721. 1926.
3. Gedye, G. R. and Allibone, T. E. Proc. Roy. Soc. Lond. 130, A: 346-366. 1931.
4. Lind, S. C., Bardwell, D. C. and Perry, J. H. J. Am. Chem. Soc. 48: 1556-1575. 1926.
5. Losanitsch, S. M. Ber. 42: 4394-4400. 1909.
6. Losanitsch, S. M. Ber. 43: 1871-1874. 1910.
7. Marshall, A. L. J. Am. Chem. Soc. 50: 3178-3197. 1928.
8. McLennan, J. C. and Glass, J. V. S. Can. J. Research, 3: 241-251. 1930.
9. McLennan, J. C. and Greenwood, G. Proc. Roy. Soc. Lond. 120, A: 283-295. 1928.
10. McLennan, J. C., Perrin, M. W. and Ireton, H. J. C. Proc. Roy. Soc. Lond. 125, A: 246-262. 1929.
11. Mund, W. and Koch, W. Bull. Soc. Chim. Belg. 34: 119-126. 1925.
12. Mund, W. and Koch, W. Bull. Soc. Chim. Belg. 34: 241-255. 1925.
13. Mund, W. and Koch, W. J. Phys. Chem. 30: 289-293. 1926.
14. Poma, G. and Bassie, G. Gazz. chim. ital. 51, II: 71-79. 1921.
15. Poma, G. and Nesti, A. Gazz. chim. ital. 51, II: 80-94. 1921.

HUMIDITY MEASUREMENTS IN THE SLIP STREAM OF FLYING AIRCRAFT[1]

BY D. C. ROSE[2]

Abstract

This paper is a report on some experimental work on hair hygrometers and the wet and dry bulb thermometer type of hygrometer used in aircraft work. The results of preliminary observations using such instruments are given, the results indicating that hair hygrometers are not satisfactory. The findings obtained can be correlated easily with the weather conditions under which the flights were taken.

In connection with some experiments on the elimination of electrostatic charging of films in cameras used for aerial photography, it was found desirable to obtain information on the relative humidity and state of ionization in the slip stream of flying aircraft where the camera is usually operated. This paper is a report of relative humidity measurements of a preliminary nature taken during four flights on Jan. 8, March 2, April 18 and June 17, 1931, each flight lasting about two and one-half to three hours.

Instruments Used

Of the several known methods of measuring relative humidity, the wet and dry bulb thermometer type of hygrometer, in spite of its many disadvantages, seemed the most suitable for the measurements required. Hair hygrometers were also used but were found to be unreliable at reduced pressures and temperatures, and chemical methods did not seem applicable. Dew point hygrometers were not tried because of the difficulty in observation and the low temperatures involved. Griffiths and Awbery (1) tried an hygrometer which depended on the measurement of refractive index of a glycerine and water mixture. It might be suitable for ordinary aeroplane use, but did not seem to give any satisfactory results at low temperatures.

The wet and dry bulb thermometers were placed side by side in a copper case with open ends in the air stream. The wet bulb was kept moist by a wick dipped in a water reservoir in the usual way. This arrangement has two great disadvantages. In the first place, in the necessarily low temperatures (the lowest encountered was $-18°$ C.), the wick freezes causing the supply of fresh water to the wet bulb to cease. Having this point in view and considering the high velocity of air about the wick, a thick coarse wick was used rather than the usual close fitting type. (See discussion of this point below.) Another difficulty involved was the reading of the thermometers. At temperatures ranging from 10° to about $-15°$ C. the difference in temperature between wet and dry bulb thermometers is so small that precise thermometers having scale divisions not greater than a fifth of a degree, or at the most a half, should be used so that tenths of a degree could be estimated. In the

[1] *Manuscript received August 26, 1931.*
Contribution from the National Research Laboratories, Ottawa, Canada.
[2] *Assistant Research Physicist, National Research Laboratories, Ottawa.*

case of liquid-in-glass instruments, thermometers with a fine bore are therefore required. For the first two flights mercury-in-glass thermometers were used having every tenth degree marked. It was found impossible to read these with any certainty, since the thermometers must be placed outside the cabin in the slip stream. A different system of measuring the temperature was therefore devised.

Instead of using mercury thermometers, a set of resistance thermometers was constructed, connected to a special bridge circuit, by means of which the temperature difference between the wet and dry thermometer could be calculated easily from the readings on a slide wire scale. A detailed description of this instrument has been published elsewhere (4). With the circuit used, the dry bulb temperature must still be read by a mercury thermometer. In the later flights the system of reading the mercury thermometers was greatly improved and instruments marked very clearly in half-degrees were used, so that it became possible to obtain comparative measures of the relative humidity with both the resistance and mercury thermometers.

The resistance thermometers were in oil-filled glass tubes having dimensions of about one-half inch by two inches. The wet bulb was covered with a thick layer of cotton and was saturated by pouring water on it about every 15 min. The thermometers were placed directly in the slip stream of the aircraft, being suspended with the other apparatus through the camera hole in a cabin monoplane.

The questions of wetting the wick, and the nature and surface of the wick are of extreme importance if precise results are to be obtained. This subject has been investigated by others (3) and it has been found that for exact results the wet bulb covering should be thin and smooth. In the present experiments the conditions were rather different, in that the temperatures were mostly below freezing. Hence the wet bulb covering was made thick so that it would hold a good layer of ice. This point will be mentioned again when the results of measurements taken with the resistance and mercury thermometers are compared. This cover as used gave quite consistent results when compared with other instruments in the laboratory.

Another problem of importance is the velocity of the air stream past the thermometers. Paine (3) gives upper and lower limits of 15 and 3 metres per second. The upper limit is due to the air at high speeds carrying away the water in spray from the wet bulb. This should have no effect in the present case, as the wet bulb was frozen most of the time. The velocity was about 30 metres per second. The reduced pressure due to altitude would also reduce the effect of too high a velocity. Paine's work was done at room temperatures and ground pressure.

The translation of the thermometer readings to relative humidity in per cent is also an important point. As far as the author is aware, there are no psychrometric tables available for readings at reduced pressures, hence a formula had to be used. Although many formulas for relative humidity have been developed, the following one (2, p. 15) seems most applicable to the present observations:

$$e = e' - 0.00066B(t - t')(1 + 0.00115t'), \qquad (1)$$

where e is the vapor pressure; e', the saturation vapor pressure at the wet bulb temperature, t'; and B, the barometric pressure in mm. of mercury; the temperatures being in degrees centigrade. The term $(1+0.00115t')$ was omitted in the present calculations because it made a negligible difference in the temperature range involved.

An "Edney" hair hygrometer was also carried during the flights—being suspended just outside the camera hole in the slip stream. The hair hygrometer was calibrated at one point in the laboratory before each flight. It is hardly expected that a hair hygrometer scale designed to work at room temperatures and ground pressures would give satisfactory results at reduced temperature and pressure. The results indicate, as was expected, that the hair hygrometer is not very good for use in aircraft.

Results

First Flight

Fig. 1 shows a summary of the results of the first preliminary flight. Both the first and second flights should be considered only as tests of the apparatus, it being understood that the results are somewhat incomplete. Owing to the way in which the readings were taken the results show up better if the observations are plotted on a time scale instead of on altitude. The pressure was read on a Tycos barometer, reading in millibars, (Curve A) carried with the other equipment, and also on the altimeter on the instrument board of the aeroplane (Curve B). The barometer readings were not converted to altitude readings. The resistance thermometers were not taken on this flight. The wet and dry bulb thermometer readings from which the relative humidity (Curve D) was obtained were unsatisfactory. The thermometers were of very fine bore, graduated in tenths of degrees and were difficult to read. Also they were not of sufficiently low range (−12° C.), so for the higher altitudes no reading could be obtained. In fact, even at

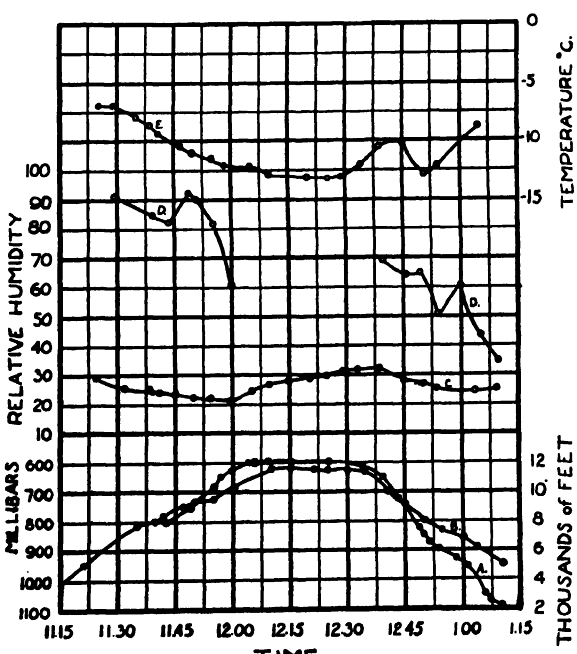


FIG. 1. *Curves representing results of the first flight. Curve A—Aircraft altimeter readings. Curve B—barometer readings. Curve C—Hair hygrometer readings. Curve D—Relative humidity from wet and dry bulb mercury thermometers. Curve E—Temperature from strut thermometer.*

the lower altitudes, owing to the difficulties in reading, the results are not very reliable. Curve C represents the readings of the hair hygrometer and E the strut thermometer attached to one of the wing struts of the plane. No exact record of the weather conditions of this and the third flight were taken at the time, but the day was clear and cloudless.

Second Flight

Fig. 2 shows a similar summary of the results of the second flight, which was made on March 2. This was the first flight on which the resistance thermometers were taken.

On this occasion the wet and dry bulb thermometers were not readable at all as the temperature was too low, but some results were obtained with the resistance thermometers. Curves A and B are the barometer and altimeter readings as in Fig. 1. It will be noted that in this and subsequent flights readings were taken at definite altitudes only, instead of during a continuous climb or descent as in Fig. 1. Owing to some trouble with the other apparatus used to measure ionization (not being reported here) the author was too much occupied to take many readings—hence the Curve C is not a complete curve but simply the humidity at the altitudes indicated. It will be noted that the second humidity observation (at 5000 ft.) is much higher than any other made during the flight. At first this was thought to be spurious but when the weather was considered, it is as would be expected. Just before the take off there was a light fall of snow and the sky was nearly overcast but clearing slowly. During the ascent there was quite a layer of clouds at altitudes around 3000 to 4000 ft. This probably accounts for the high humidity at 5000 ft. During descent most of the cloud bank was gone and, in fact, the descent took place over a considerable area which was entirely free from clouds, so no such increase in relative humidity was noticed. This increase in relative humidity due to the clouds was not noticed on the hair hygrometer (Curve D). Curve E is the temperature as measured by a strut thermometer.

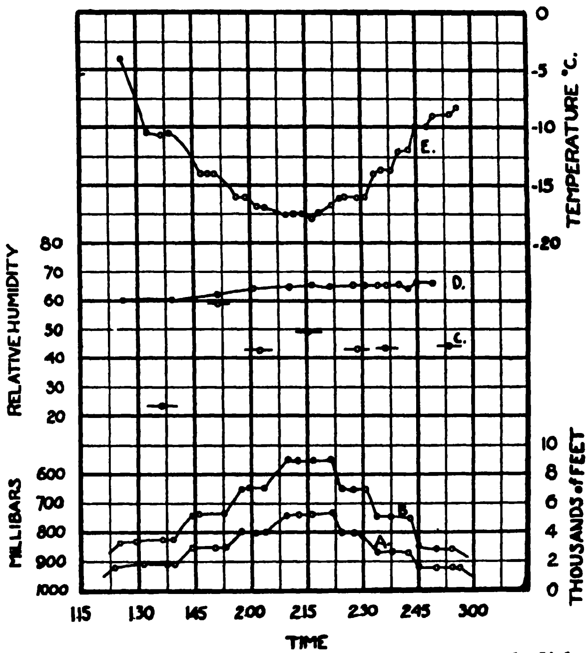


FIG. 2. *Curves representing results of the second flight. Curve A — barometer readings. Curve B—Aircraft altimeter readings. Curve C—Relative humidity from wet and dry resistance thermometers. Curve D—Hair hygrometer readings. Curve E—Temperature from strut thermometer.*

Third Flight

Fig. 3 represents the results of the third flight on April 18. For this flight a new set of mercury thermometers had been obtained, having every half-degree clearly marked and of lower range so that they could be read easily. Following along the time scale as one ascends, the temperature drops. The total moisture content of the air also drops, but relatively not as fast as the temperature, so the relative humidity increases to nearly 100% (Curve C). Then, ascending further, the temperature does not drop as rapidly and, since the moisture content is still decreasing, the relative humidity decreases. During the descent the process is reversed. No exact record of the weather was kept but it was either a clear or nearly clear sky, with a few fairly low cumulus clouds. The air was very bumpy at altitudes up to 4000 ft., making it difficult to take readings. The point marked X on Curve D is probably spurious as the water on the wet bulb thermometer was just freezing, making this thermometer temperature reading higher than it should be. This trouble always occurs near the freezing point when wet and dry bulb thermometers are used. The fact that the mercury thermometers (see Curve D) did not give the same results as the resistance thermometers is obviously due to unsatisfactory wetting of the bulb of the former, due to the freezing of the wick as previously explained. The wet resistance thermometer was saturated at every 2000-ft. level during the ascent, but as the supply of water was accidentally spilled when at the greatest height, it was not wet during descent. The consequence is that the humidity peak on descent is not as pronounced as during ascent. Care was taken not to take any readings immediately after wetting the wet thermometer, so that the temperature would have time to come to equilibrium.

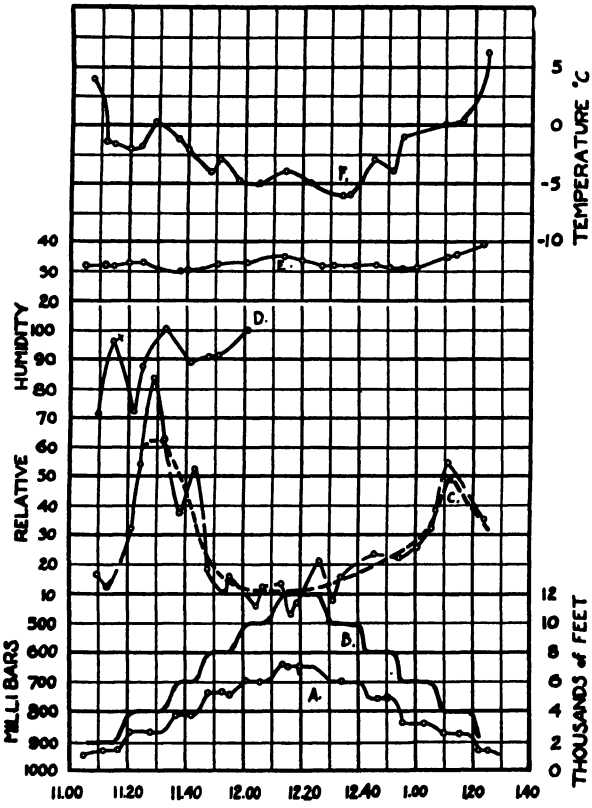


FIG. 3. *Curves representing results of the third flight. Curves A, B and C—as in Fig. 2. Curve D—Relative humidity from wet and dry mercury thermometers. Curve E—Hair hygrometer readings. Curve F—Temperature from dry mercury thermometer.*

The broken curve represents a probable average smooth curve, the sudden variations which are due to local air currents being omitted. One would expect the humidity curve to have variations in a reverse direction from those in the temperature curve, that is, as the temperature drops, relative humidity should rise and *vice versa*. To some extent this is so and a closer agreement would probably have been obtained were it not for the fact that the resistance thermometers show a lag greater by a few minutes than that for the dry mercury thermometer.

Curve E represents the readings of the hair hygrometer. It does not show the variations in humidity in agreement with the other instruments. Curve F shows the temperature read on the dry bulb thermometer in the slip stream.

Fourth Flight

Fig. 4 represents the results of the fourth flight made on June 17. The results obtained during this flight are more complete than any other. Readings were obtained throughout the flight of wet and dry bulb temperatures, both with the resistance and mercury thermometers. Curves A and B as usual represent the barometer and aircraft altimeter readings. In this flight, an altitude of 15,000 ft. was reached, whereas previously 13,000 ft. was the highest.

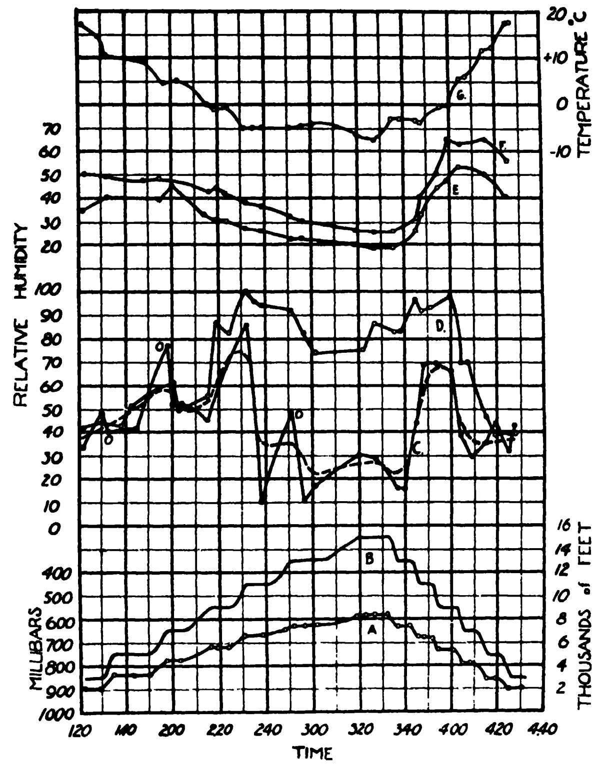


FIG. 4. *Curves representing results of the fourth flight. Curves A, B and C—as in Fig. 2. Curve D—Relative humidity from wet and dry mercury thermometers. Curve E—Hair hygrometer (same instrument as used in Flights 1, 2 and 3). Curve F—Hair hygrometer (belonging to Department of National Defence). Curve G—Temperature from dry mercury thermometer.*

Following the course of this flight: as the temperature dropped, the relative humidity (see Curve C) rose, showing two maxima—one small peak around 7,000 ft. and a large one at 9,000 and 11,000 ft. The relative humidity was rising while at the 9,000-ft. level and descending rapidly while at the 11,000-ft. level. The smaller peak around 7,000 ft. appears because of a drop in relative humidity while at the 7,000-ft. level, corresponding to a slight reversal in the temperature curve.

The curves representing relative humidity as measured with the wet and dry

bulb mercury thermometers (Curve D) followed closely results derived from measurements with resistance thermometers (Curve C) until about the freezing point, when the mercury thermometers indicate a higher humidity due no doubt to inadequate wetting of the wet bulb by the wick and reservoir method.

The relative humidity at altitudes higher than 11,000 ft. goes down considerably. Then during descent the process is reversed—a large increase in relative humidity appearing at the 9,000- and 11,000-ft. levels. This is exactly the sort of curve one would expect when the weather is considered. The day was clear except for numerous cumulus clouds at altitudes between 7,000 and 9,000 ft. The points marked "O" on Curve C are not as reliable as the others, as they were taken either when the wet bulb was just freezing or too soon after it had been saturated with water. They are probably higher than they should be. The broken curve represents an average smooth curve as in Fig. 3.

Curves E and F represent readings taken on hair hygrometers—E being read on the same hygrometer used in Flights 1, 2 and 3. An additional hair hygrometer belonging to the Department of National Defence was carried on this flight, the results being shown by Curve F. The relative humidity recorded from the hair hygrometer readings does not follow that calculated from the wet and dry bulb thermometer. On the descent an increase in relative humidity is shown corresponding to that shown on Curve C, but with considerable lag.

Curve G represents the temperature in the slip stream measured by the dry mercury thermometers.

Conclusions

The results indicate that the hair hygrometer is not satisfactory for use in aircraft work. Of the other two instruments used the resistance thermometers gave the better results, but no doubt equally good results could be obtained with mercury thermometers if an improved method of wetting the bulb were developed, and the thermometers so mounted that they could be easily read. In Fig. 4, the author has assumed that the resistance thermometers (Curve C) gave the more accurate results because the mercury thermometers had proved unsatisfactory in previous work.

The accuracy of the measurement is not very great and, though in fine weather the results indicate a low humidity (10 to 30%), at altitudes of 10,000 to 15,000 ft. there is a little uncertainty as to the correctness of this value because of the uncertainty in the accuracy with which the formula (1) can be used. Also, as stated above, the high velocity of ventilation and the rough wicks used may have some effect on the accuracy. However, owing to the consistent way in which the instrument behaved in the laboratory, it is not expected that the error is very great.

The experiments indicate that some rapidly responding, accurate and rugged form of instrument for measuring humidity is badly needed for aircraft work.

Relation to Weather

The results of these experiments, particularly the last two flights (Fig. 3 and 4), show definite agreement with what would be expected. As one ascends

to the cloud level, the relative humidity goes up nearly to 100%. One would expect that it should be 100% in, or in the neighborhood of, clouds. During these flights the aeroplane was not flown through or very near any clouds—which probably accounts for the fact that 100% humidities were not found except in cases where the readings of the thermometers were uncertain.

On a subsequent flight made by the author, carrying an aerometrograph only, which contains a hair hygrometer, the plane was flown through clouds for several minutes. The hair hygrometer showed an increase in humidity of about 15%, but not going above 60%. This is further evidence of the unsatisfactory nature of hair hygrometers for aircraft work.

Another interesting feature of these experiments was the altitude of the point at which maximum relative humidity occurred. In the two flights taken in cloudy weather (Fig. 2 and 4) the point of maximum relative humidity appeared to be a considerable distance above the clouds. This is particularly definite in Fig. 4. On this flight the clouds were between 7,000 and 9,000 ft. high, while the relative humidity maxima were found to be between 9,000 and 11,000 ft., both on ascending and on descending. In Fig. 2, the clouds were very low (about 3,000 ft.) and though the results here are not so complete, the maximum humidity recorded was at 5,000 ft. This may be due to the fact that during the flying, clouds were avoided as much as possible. This high recorded position of the point of maximum humidity, and the fact that 100% humidities were not recorded at all may possibly have been due to the location of the instruments in the aeroplane. It is quite possible that the air in the slip stream was slightly warmer than the atmosphere surrounding the plane because of heat from the engine. In fact in Flight 3 it was noticed that the temperature as read on the strut thermometer was about 2° lower than that read on the thermometer in the slip stream. This, however, was not the case in Flight 4, so it may have been due to the strut thermometer being inaccurate in Flight 3.

It is expected that further work of this nature will be undertaken by the author with improved equipment.

Acknowledgment

The author wishes to thank the officers of the Department of National Defence—particularly the pilots who undertook the flying—for their co-operation in this work.

References

1. Griffiths, E. and Awbery, J. H. Proc. Phys. Soc. London, 39: 79-84. 1926.
2. Humphreys, W. J. Physics of the air. 2nd ed. McGraw-Hill, 1929.
3. Paine, G. P. Proc. Natl. Acad. Sci. 11:555-562. 1925.
4. Rose, D. C. Can. J. Research, 5: 156-161. 1931.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL *of* CANADA

VOLUME 5 | NOVEMBER, 1931 | NUMBER 5

COMPARISON OF COMPOSITES AND AVERAGES WITH RESPECT TO BAKING QUALITY

I. PURE SAMPLES OF ONE VARIETY[1]

BY R. K. LARMOUR[2] AND S. F. BROCKINGTON[3]

Abstract

Comparisons of the loaf volume of composite samples with the average loaf volume of the individuals comprising the composite were made on three groups of samples: namely, (1) samples of pure Marquis grown in one locality in one season, grouped on the basis of protein content; (2) samples of pure Marquis, Reward, and Garnet in separate series grouped on basis of protein, irrespective of locality of origin; and (3) samples of the above three varieties grouped on basis of origin, irrespective of protein content.

In these studies the composite samples were all made up after the individual flours had been baked and, therefore, there was admitted an error due to the time factor in respect to age of the flours and also in respect to variability in baking technique. Despite this, however, there was found a very close agreement between the values obtained with the composite samples and the average values of the component flours. There were a large number of cases in which there was practically complete agreement between the two values and only a few in which the differences were very great. The correlation of the two values was on the average of the order of $+0.95$ and the conclusion was reached that the data obtained with the composite samples could be used safely as an estimate of the average values and *vice versa*.

The question whether or not a composite sample of wheat or flour gives as accurate information regarding baking quality as could be obtained by taking the average values of a number of individual tests, is one of very considerable interest to cereal chemists. If it could be demonstrated that the quality of a composite made up of twenty different samples of No. 1 Northern Marquis wheat were equal to the mean value of the individual tests, an enormous amount of time and labor might be avoided in certain types of investigation. As an instance we might consider the comparison of five varieties of hard red spring wheat. The ordinary procedure is to obtain samples of these varieties grown in many different localities, mill and bake them individually and then

[1] *Manuscript received September 21, 1931.*
Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Canada. Issued as Paper No. 18 of the Associate Committee on Grain Research, National Research Council of Canada.

[2] *Assistant Professor of Chemistry, University of Saskatchewan.*
[3] *Graduate student, University of Saskatchewan.*

take the average of the results so obtained as a measure of the relative value of the respective varieties. If we could be certain that mixing the individuals of one variety would result in no complementary action, and provided we were not interested in comparing degree of variability, it would be much simpler to mix all samples of each variety, making thus five composite samples.

The cereal chemist who judges the quality of a wheat crop in a given area by reference to the average of his tests of individuals, tacitly assumes that the average value is an estimate of the value of the composite because he knows that the separate lots tested will eventually be made into composites, represented by the cargo, to be sold and used as such.

On the other hand, cereal chemists often judge the average value of a crop by the use of composites. Each year in Western Canada the quality of the crop is estimated and the relative value of the grades determined by testing the so-called "average" samples collected at various inspection points. These "average" samples are in reality composites made up by combining small aliquots according to the grade. If all the carloads of wheat represented in the composite sample were thoroughly mixed together and cargo shipments were made from that one bulk, doubtlessly the milling and baking test would give a reliable estimate of the particular class of wheat. This, however, does not occur because the car lots represented in the "average" become assigned to various elevators and go into various combinations to make up different cargoes. In using the quality as determined on the inspection point "averages" to judge the whole crop, therefore, there must be made the assumption that the quality of the composite fairly represents the average quality of the number of separate parcels that may be made up from the whole class. To put the case more concretely; suppose an "average" No. 1 Northern sample from Winnipeg

TABLE I

A COMPARISON OF SOME COMPOSITES AND AVERAGES

50% Hard red spring wheat flour plus 50% of	Loaf vol. cc.	Average L.V. of individuals	Composite as % of average
Australian flour	635	622	102
English flour	562	560	100
Durum of 1927	600	572	105
Durum of 1928	590	510	116
Durum of 1929	645	585	110

is made up of portions of 1,000 cars of that grade. These 1,000 cars conceivably might be used in making up five separate composite cargoes of No. 1 Northern wheat. To judge these parcels by the quality of the original "average" sample would mean assuming that it represents the average of samples obtained from the respective cargoes. In other words, it is taken for granted that there is very little if any complementary action when various lots of the same class of wheat are mixed together.

Thus we have on one hand, estimates of composites being made from averages, and on the other, estimates of averages being made from composites.

If the same conclusions can be reached by either procedure, it obviously would be very advantageous in many instances to choose the use of composites on account of the saving of labor.

Aside from the loss of information regarding variability, the principal objection to composites has been the belief that in mixture the component flours tend to complement each other and give results essentially different from the averages of the individuals. It can be easily demonstrated that this takes place in certain types of mixture and not in others. A few cases are given in Table I.

With the Australian flour there is evidence of a slight difference between the composite and the average of the components. With the durums the difference is quite marked and it varies with the different crops. With the flour milled from native English wheat there is no apparent difference between the composite and the average values. The greatest difference is found, in the cases cited, when flours from those wheats having the most diverse characteristics are mixed. The problem is to ascertain where to draw the line between wheats that may and may not be mixed without this complementary effect occurring. Can we safely mix samples of one variety, or of one class, or wheats from a given area, or only wheats of a certain protein content? In order to find an answer to these questions we have started with the simplest sort of mixtures and from there tried mixtures that promised to be more and more incompatible, or more likely to give complementary effect. As the first phase of this investigation there has been made a comparison of composites and averages of samples of a single pure strain grown in a very limited area and grouped on the basis of protein content.

Pure Samples of One Variety Grown in One Locality

The material consisted of 98 samples of pure strain Marquis wheat grown in one locality. These were milled on an Allis-Chalmers experimental mill to a straight flour representing about 95% of the total. After aging in cotton sacks for two months they were baked by the basic and bromate formulas with certain modifications described by Larmour and MacLeod (2).

TABLE II

COMPARISON OF COMPOSITES AND AVERAGES OF PURE STRAIN MARQUIS SAMPLES GROWN IN ONE LOCALITY

N.	Protein class, %	Basic L.V., cc.			Bromate L.V., cc.		
		Composite	Average	Diff. C-A	Composite	Average	Diff. C-A
4	9.0 - 10.9	550	548	2	565	586	−21
12	11.0 - 11.9	538	522	16	590	603	−13
24	12.0 - 12.9	560	538	22	630	646	−16
16	13.0 - 13.9	560	559	1	673	706	−33
17	14.0 - 14.9	572	573	−1	775	782	− 7
22	15.0 - 15.9	582	574	8	770	789	−19
3	16.0 - 16.9	593	598	−5	825	841	−16

After baking, these samples were stored in tightly covered tin cans for several months. The composites were then made up by combining flours from wheats of similar protein range. They were classified according to wheat rather than flour protein because our other studies of the relation of protein and quality have been made on this basis, and it therefore proved to be the more convenient. The combined flours were very thoroughly mixed, sampled and then baked in triplicate. Table II gives the values obtained for the composite flours together with the average values calculated from the individual tests. The data are shown graphically in Fig. 1.

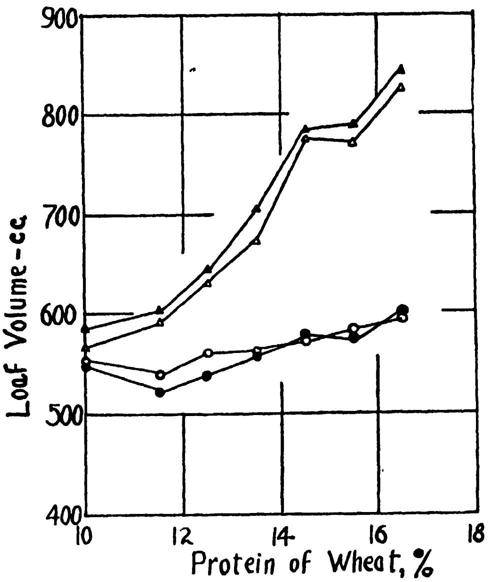


FIG. 1. *Comparison of composites and averages of pure strain Marquis samples grown in one locality.* ▲—*Average loaf volume by bromate formula.* △—*Composite loaf volume by bromate formula.* ●—*Average loaf volume by basic formula.* ○—*Composite loaf volume by basic formula.*

Comparison of the curves for the basic data indicate that the results are in very close agreement, the widest variation being 22 cc. found in the class, 12.0—12.9%. The mean difference is 6 cc. in favor of the composite.

With the bromate data there is a fairly consistent difference between the two sets of results, the composite being lower than the average by a mean difference of 18 cc. There is, however, a closer relationship between the bromate values than between the basic values. This is shown by the respective correlation coefficients.

	Basic	Bromate
$r_{a.c.}$ =	$+0.9458$	$+\ 0.9969$

From Table V. A., Fisher (1), $P = 0.01$ when the value of $r = 0.8745$. These coefficients may therefore be considered significant.

With the bromate formula there appears to be a quite constant difference between loaf volume of the composites and the average loaf volumes. It can scarcely be attributed to change in baking technique as the results by the basic formula do not support such a supposition. It might represent a uniform decrease in strength due to age, or some sort of complementary effect resulting from mixing. No definite answer to this question is forthcoming at present.

From the results of this study it has been concluded that for comparing classes of one strain of wheat grown in a limited locality, the composite samples yield practically the same relative values as those obtained by calculating class averages from the tests of individuals. If any complementary effect occurs

it is slight and certainly not great enough to lead to any serious error in estimating the relative average value of the various classes.

Pure Strain Samples of One Variety Grown in Various Localities and Grouped on the Basis of Protein

Having shown that samples of one variety grown in one locality show good agreement between the loaf volume of the composites and the average loaf volume of the individuals, the next step was to examine this relationship for samples grown in widely differing localities. It is known that environment affects the quantity of protein but we do not know if in one variety there would be qualitative differences sufficiently great to produce complementary effect when the different flours are mixed together.

In this study there were used 138 samples of Marquis, 83 samples of Reward and 89 samples of Garnet, respectively, in three series. All samples were grown in Saskatchewan in the season of 1929. As in the previous study the grouping was based on protein of wheat using increments of 1%. The average loaf volumes and loaf volumes of the composites as well as the baking scores for the three series are given in Table III and shown graphically in Fig. 2, 3 and 4.

It can be seen that these data differ from those previously discussed in one important aspect, namely, that the values for the composites are not consistently lower than the averages. This indicates that the differences are

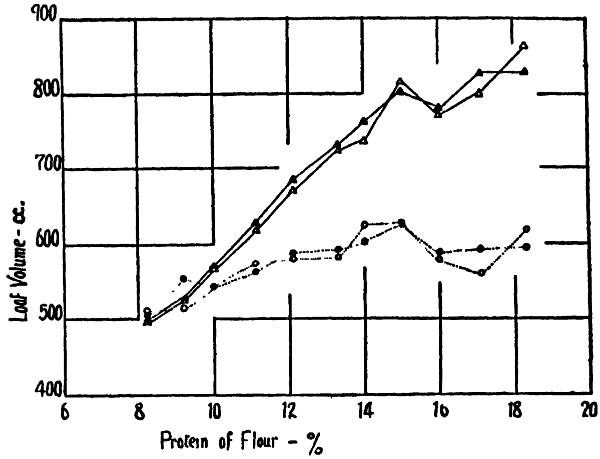


FIG. 2. *Comparison of composites and averages of Marquis samples grouped on basis of protein irrespective of locality.*—▲, △, ●, ○—*as in Fig. 1.*

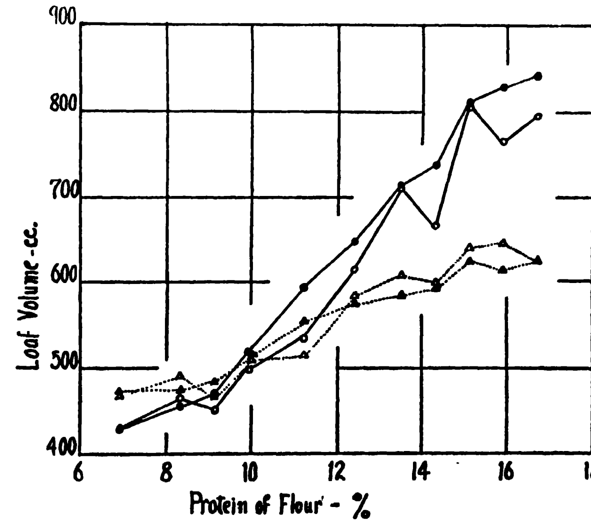


FIG. 3. *Comparison of composites and averages of Garnet samples grouped on basis of protein irrespective of locality.*—▲, △, ●, ○—*as in Fig. 1.*

TABLE III

COMPARISON OF AVERAGES WITH COMPOSITES GROUPED ON PROTEIN BASIS IRRESPECTIVE OF LOCALITY OF ORIGIN

Wheat protein class, %	No. in comp.	Protein of flour composite	L.V. Basic		C.B.S. Basic		L.V. Bromate		C.B.S. Bromate	
			Ave.	Comp.	Ave.	Comp.	Ave.	Comp.	Ave.	Comp.
Marquis										
8–9	6	8.2	504	505	62	60	500	495	67	66
9–10	5	9.2	554	515	73	68	526	525	70	77
10–11	11	10.0	545	543	76	75	569	568	89	88
11–12	15	11.1	563	573	82	87	627	618	101	100
12–13	12	12.1	588	580	85	87	686	670	113	112
13–14	28	13.3	593	583	86	86	731	725	122	124
14–15	22	14.0	604	625	89	94	762	735	128	126
15–16	14	15.0	626	628	91	92	803	815	136	140
16–17	12	16.0	588	580	84	84	779	770	131	131
17–18	8	17.1	592	560	90	77	827	800	141	137
18–19	5	18.3	595	618	83	90	827	865	136	149
Reward										
9–10	3	8.9	532	540	68	66	498	495	60	55
10–11	4	9.9	540	530	76	70	550	548	82	76
11–12	6	11.0	558	548	82	75	611	603	95	94
12–13	7	12.1	586	598	88	86	694	665	115	109
13–14	10	13.1	608	588	91	86	717	735	121	123
14–15	11	14.4	609	620	85	90	798	793	135	134
15–16	19	15.4	647	673	96	102	837	880	142	151
16–17	13	16.3	665	700	99	109	883	930	152	160
17–18	7	17.4	674	728	105	117	937	913	159	158
18–19	3	17.9	645	655	94	96	933	945	154	164
Garnet										
7–8	5	6.9	470	468	47	50	428	428	37	39
8–9	7	8.3	472	490	50	55	455	465	45	44
9–10	8	9.1	484	468	58	54	470	448	56	46
10–11	12	9.9	516	510	63	66	519	498	68	65
11–12	7	11.2	554	515	78	68	594	535	92	82
12–13	12	12.4	574	583	78	88	648	613	104	100
13–14	13	13.5	585	608	80	87	713	710	117	122
14–15	10	14.3	596	598	85	83	737	665	123	114
15–16	7	15.1	625	640	91	94	813	805	136	141
16–17	6	15.9	614	645	92	96	830	765	140	133
17–18	2	16.7	625	625	94	94	842	795	141	134

TABLE IV

CORRELATION BETWEEN THE AVERAGE LOAF VOLUME AND LOAF VOLUME OF THE COMPOSITE

Variety	N.	r Basic	r Bromate	1% point* of r
Marquis	11	+0.888 ±0.043	+0.989 ±0.004	0.735
Reward	10	+0.975 ±0.011	+0.989 ±0.004	0.765
Garnet	11	+0.964 ±0.014	+0.986 ±0.006	0.735

**Table V.A., Fisher (1).*

probably due to experimental error to a greater degree than in the other series. This may be accounted for by the fact that less time elapsed between the baking of the composites and the individuals.

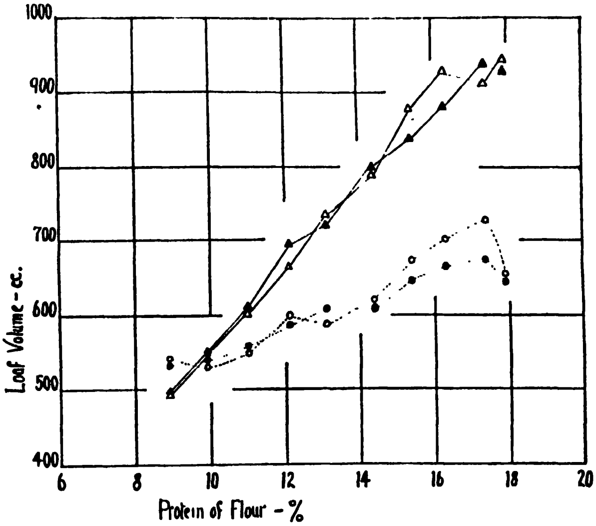


FIG. 4. *Comparison of composites and averages of Reward samples grouped on basis of protein irrespective of locality.* ▲, △, ●, ○—*as in Fig. 1.*

In order to show more clearly the close relationship of the composite and average bromate values we have plotted the one against the other in Fig. 5. As the same units are used on both axes, a line of 45° slope would indicate equality of the two sets of values. This has been drawn in and the data actually show a close approximation to this curve. It is evident that prediction of the average based on the values obtained with composite samples would not be greatly in error for the whole range of protein studied.

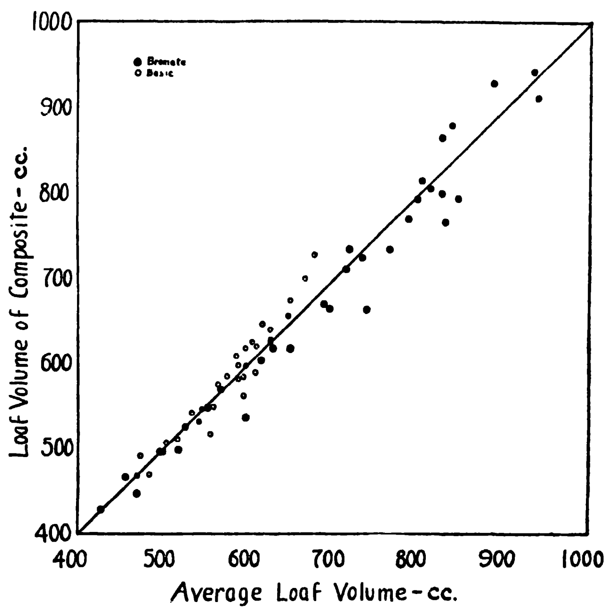


FIG. 5. *Comparison of composites and averages of Marquis, Reward, and Garnet samples grouped on basis of protein.*

The correlation coefficients are given in Table IV and they indicate very close relationship of the two sets of values. It seems justifiable, therefore, to conclude that with sound samples of a given pure variety of one season, the loaf volume of the composite samples may be regarded as an excellent estimate of the average values to be expected and *vice versa*.

Pure Strain Samples of One Variety Grouped on Basis of Locality of Origin

In both studies heretofore considered the samples were grouped on the basis of protein content of wheat. As it is generally considered that environmental

TABLE V

COMPARISON OF AVERAGES AND COMPOSITES GROUPED ON BASIS OF AREA OF ORIGIN

Area	No. in composite	Protein of flour composite	L.V. Basic			L.V. Bromate		
			Ave.	Comp.	Diff. A-C	Ave.	Comp.	Diff. A-C
Marquis								
I	8	11.8	537	535	2	604	593	11
II	14	10.2	545	548	−3	571	568	3
III	9	13.6	614	608	6	783	755	28
IV	25	14.2	599	593	6	741	710	31
V	9	16.2	603	602	1	785	795	−10
VI	16	15.8	598	613	−15	795	815	−20
VII	14	13.9	594	588	6	756	765	− 9
VIII	12	12.4	598	590	8	698	670	28
IX	10	13.2	583	590	− 7	709	730	−21
X	10	13.2	604	603	1	707	700	7
Reward								
I	12	13.1	599	635	−36	708	695	13
II	14	11.9	577	568	9	652	628	24
III	6	14.5	611	603	8	796	773	23
IV	11	15.5	648	670	−22	843	878	−35
VI	7	17.4	639	653	−14	869	868	1
VII	7	15.5	649	650	− 1	852	868	−16
VIII	11	13.9	614	623	− 9	770	768	2
IX	6	15.3	623	700	−77	815	853	−38
X	9	15.3	659	643	16	827	818	9
Garnet								
I	12	10.2	513	505	8	533	490	43
II	16	8.6	495	485	10	475	443	32
III	7	12.1	569	558	11	665	598	67
IV	9	13.5	597	573	24	718	645	73
VI	3	16.1	613	620	− 7	825	838	−13
VII	8	13.0	596	603	− 7	707	668	39
IX	4	13.2	575	615	−40	720	695	25
X	8	12.4	568	593	−25	660	638	22

TABLE VI

CORRELATION BETWEEN LOAF VOLUME OF THE COMPOSITE AND THE AVERAGE LOAF VOLUME FOR THE SERIES GROUPED ON BASIS OF ORIGIN

Variety	N.	r		1% point* of r
		Basic	Bromate	
Marquis	10	+0.961	+0.971	0.765
Reward	9	0.657	0.976	0.798
Garnet	8	0.913	0.978	0.834

**Table V. A., Fisher (1).*

conditions profoundly affect the strength of wheat, one might suppose that samples having the same protein content might have been grown under nearly the same environmental conditions, and that therefore they would show similar characteristics. The observation that composites check the average values so closely, confirms this supposition. In order to obviate this possibility, a number of composites were made up on the basis of locality of origin. The samples at hand were divided into ten groups representing as many areas into which the province had been subdivided. Comparison of loaf volumes of these composites and the respective average values is made in Table V and shown graphically in Fig. 6.

The agreement between averages and composites is quite good for Marquis, by both the standard and bromate formulas. The Reward gives quite close agreement except in the case of Sample 9 which is 77 cc. greater than the average value for the basic baking. The Garnet basic results are not any too close but the agreement is good enough for the purpose of predicting one value from the other with a reasonable degree of accuracy. The Garnet bromate data show four cases of very poor agreement. It should be noted too that in all but one case the average value was greater than the value obtained with the composite. No reasonable explanation for this is apparent.

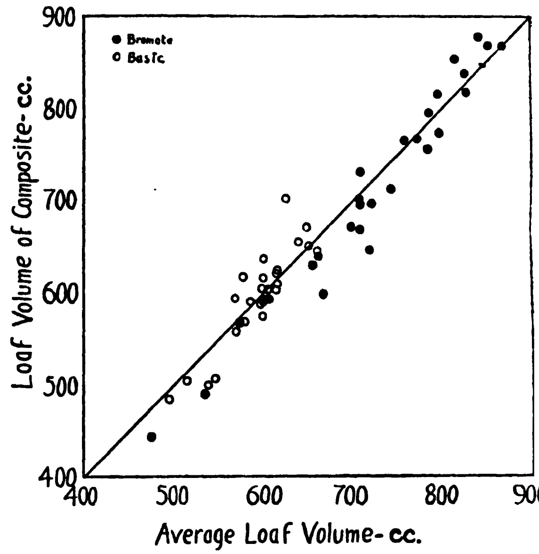


FIG. 6. *Comparison of composites and averages of Marquis, Reward, and Garnet samples grouped on basis of area of origin.*

As can be seen from Fig. 6, the relationship between composites and average values is very close. The correlation coefficients for the three varieties are given in Table VI. The value +0.657 for the Reward basic data is not significant, due to the wide discrepancy in values for Sample 9, but all the others show a high degree of correlation, indicating that the two variables are very interdependent. It appears safe, therefore, to conclude that either the composite or average loaf volume may safely be used to predict the other with a reasonable degree of accuracy.

This means that in area surveys for a single season, where one is dealing with one variety of wheat, a very great amount of time and work can be avoided. In place of milling and baking hundreds of samples, and calculating the average values, composites consisting of aliquot portions can be prepared and tested to give information as adequate as obtainable from the average of the individual tests. The use of the composite sample recommends

itself furthermore inasmuch as, with the great reduction of samples to be handled, many more tests can be made and the net result will be a more complete and comprehensive estimate of the character of the wheat in hand.

References

1. Fisher, R. A. Statistical methods for research workers. 3rd ed. Oliver and Boyd, London. 1930.
2. Larmour, R. K. and MacLeod, A. G. Application of the bromate differential test in the estimation of baking quality of Canadian hard red spring wheat flour. Sci. Agr. 9: 477-90. 1929.

VARIETAL TRIALS, PHYSIOLOGIC SPECIALIZATION, AND BREEDING SPRING WHEATS FOR RESISTANCE TO *TILLETIA TRITICI* AND *T.LEVIS*[1]

By O. S. Aamodt[2]

Abstract

There has been a considerable increase in the amount of bunted wheat in western Canada recently. One hundred and forty-nine varieties and selections of spring wheat showed all gradations in reaction to this disease when inoculated artificially, varying from apparent immunity to high susceptibility. The increase in bunt can be accounted for in part by the use of certain varieties that are more susceptible than some of those grown formerly. There has also been an increase in the number and virulence of physiologic forms. One physiologic form of *T. tritici* and five of *T. levis* were obtained from six collections of bunt in this study. The isolation and study of relatively pure forms of the organism will be necessary for a study of the genetic factors in the host governing the reaction to bunt. Inheritance studies at present indicate that multiple factors, the exact nature of which has not yet been determined, govern the reaction to this disease. Production of resistant varieties suitable for the prairie provinces of Canada offers a very promising means for reducing the losses due to bunt of wheat.

Introduction

There is a great need in western Canada for improved varieties of hard red spring wheats that are resistant to such destructive diseases as rust, foot- and root-rots and smut. In the province of Alberta destructive epidemics of stem rust are very infrequent but the foot-rots and smuts are more destructive as well as more frequent in occurrence. In the wheat improvement program at the University of Alberta these latter diseases are being given primary consideration.

Bunt, or stinking smut, of spring wheat caused by *Tilletia tritici* (Bjeck.) Wint. and *T. levis* Kühn appears to be increasing rapidly in western Canada in recent years (22). This increase in prevalence may be attributed to several causes. First, the growing of varieties of wheat that are more susceptible to bunt than some of the older standard varieties such as Marquis. Second, an increase in the number, prevalence and distribution of more virulent strains of the bunt organism. Third, the failure of farmers to treat their seed regularly, or the practice of treating carelessly and inefficiently. Fourth, variation in quality of the fungicides and the consequent ineffectiveness of the treatments. Data concerning the first two of the above-mentioned causes of the increase in bunt are presented in this paper. A preliminary report on the results obtained in an attempt to produce resistant varieties by hybridization is also included.

In many sections of the wheat growing areas on this continent the heavy annual losses of wheat and the lowering of commercial grades by bunt point to the need for more certain means of bunt control. The possibility of seed injury under certain conditions, and the expense of seed treatment would be largely eliminated through the use of resistant varieties. The growing of

[1] *Manuscript received September 15, 1931.*
Contribution from the Department of Field Crops, University of Alberta, Edmonton, Canada, with financial assistance from the National Research Council of Canada.

[2] *Professor of Genetics and Plant Breeding, University of Alberta.*

wheats immune from bunt would insure greater production without materially increasing the cost.

The first step in the production of resistant varieties is to test all available varieties with the hope of finding some that are naturally resistant to bunt. The second step is to select as parents for crossing such varieties and strains as are most likely to produce progeny some of which are both resistant to bunt and desirable commercially. Owing to the need for the development of varieties resistant to all diseases that are likely to be limiting factors in production, the second method is proceeded with directly on the basis of the knowledge available from other breeding studies. The synthetic production of new varieties through the use of composite crosses seems to be the logical method of attack upon this complicated problem. General knowledge of the genetics of wheat, and of the mode of inheritance of the characters being dealt with in particular, are greatly needed. Such information would enable one to plan more intelligently and definitely the method of attack and would insure greater success in the production of varieties with particular recombinations of characters.

Varietal Reaction

Extensive studies have been made on bunt resistance, especially of winter wheats. These studies have shown that there are wide differences in susceptibility between varieties. Woolman and Humphrey, 1924 (44), have summarized the early literature on varietal reaction to bunt. They report N. E. Tscharner as the first to note differences in varietal reaction to bunt as early as 1764. In 1901, William Farrer (13) reported the results obtained from inoculating ten varieties of wheat. Numerous observations and studies have been made since that time. More recently Vavilov, 1918 (43), Heald and Woolman, 1915 (25), Gaines, 1918-23 (17, 18, 19), Stephens and Woolman, 1922 (41), Johnston, 1924 (28), Coons, 1924 (9), Reed, 1924 (32), Faris, 1924 (11), Tisdale *et al.*, 1925 (42), Briggs, 1926 (4), Sampson, 1927 (39), Reichert, 1928 (36), and Heald and Gaines, 1930 (24), have made extensive studies on the reaction of wheat to bunt. These workers have dealt primarily with winter wheats.

Reports on the differential reaction of spring wheats, especially the hard red spring wheats, are comparatively meagre. Stakman, Lambert and Flor in 1924 (40), reported on an extensive study conducted over the five-year period 1919-1923. Their studies were on the reaction of spring wheats to *T. levis.* Rodenhiser (38) continued these studies and reported on the results obtained during the years 1924-1927. In 1929, Brentzel and Smith (2) reported on the varietal reaction of a number of spring wheats to both *T. tritici* and *T. levis.* In 1930, Holton (26) reported on the reaction of eight wheat varieties to *T. tritici.*

Stakman, Lambert and Flor (40) point out that bunt has been relatively unimportant in the hard red spring wheat area because of the resistance of the commonly grown varieties. Haynes' Bluestem and Preston wheats preceded Marquis and were "somewhat resistant." Marquis was fairly resistant

to their collection of *T. levis*, the highest per cent infection obtained being nine. Rodenhiser (38) found also that these varieties were fairly resistant. Kota was quite susceptible and it was pointed out that if Kota should be grown generally on account of its resistance to stem rust, the importance of the bunt problem would probably be increased. These investigators found that when working with *T. levis* it was evident that the durum wheats (*Triticum durum*) as a class were resistant.

In the spring wheat region of the United States a heavy epidemic of bunt occurred in 1924 resulting in an enormous loss of grain and lowering of the grade. Smut infested seed was evidently used by the growers in 1925 and 1926 with subsequent heavy losses. One of the unusual features of these epidemics was that durum wheats as a group were being attacked heavily. The results of Stakman, Lambert and Flor (40) as well as earlier observations in the field had indicated that durum wheats as a group were resistant. Samples of smutty wheat from the grain terminals at Minneapolis and Duluth were examined for their spore contents and it was found that almost all shipments of smutty, amber, durum wheat contained *T. tritici*, while only a few contained *T. levis*. The reverse was true of the hard red spring wheats where most of the smutty shipments of wheat contained *T. levis* and only a few contained *T. tritici*.

Güssow and Conners (21), in 1927, reported the results of a survey made from 27 samples of smutty wheat representing all the grain growing sections of the prairie provinces of Canada. They found that ten of the 27 samples contained mostly *T. levis* spores and originated south of a line running east and west between Winnipeg and Calgary. The other 17 samples contained mainly *T. tritici* and were found north of this line. Results of a similar nature, although not as marked, were reported by Hanna and Popp in 1930 (22).

Reichert (36), in Palestine, found that the durum wheats were not more resistant to bunt than the common wheats as was commonly held. They were actually more susceptible than the common wheats to the forms of *T. tritici* present in Palestine.

In a recent publication from North Dakota, Brentzel and Smith (2) demonstrate clearly the relationship between the two species of bunt on wheat and the commonly grown varieties of durum and bread wheats. The durums as a group were fairly resistant to *T. levis* as reported by Stakman, Lambert and Flor but were very susceptible to *T. tritici*. The reverse was true of most of the commonly grown hard red spring wheats. Kota, Ceres and Progress were highly susceptible to both species of bunt.

Holton (26), in 1930, demonstrated also that the increase in severity of bunt on durum wheats could be explained by the presence of unusually virulent strains of *T. tritici*.

Owing to the severity of black stem rust epidemics in Manitoba there has been a great increase in the percentage of the wheat acreage devoted to the growing of durum wheats, these wheats, in general, being less severely injured by stem rust. In 1928, 16.5% of the durum wheat graded smutty (22). Here

again the durum wheats were found to be infested almost entirely with *T. tritici*. This instance provides a clear demonstration of how a new variety may greatly aggravate the losses due to a particular disease because of its greater susceptibility to the causal organism.

Several varieties of common wheat have been introduced also into the hard red spring wheat area of Canada and the United States in an attempt to reduce the losses due to black stem rust. These are Kota, Ceres and Progress. They are all somewhat resistant to stem rust but in addition to being susceptible to *T. levis* like the older varieties of common wheat they are also highly susceptible to *T. tritici*. These new common wheat varieties then, together with the durum wheats, have greatly aggravated the bunt problem, not only because of the inability of the varieties to withstand attacks by the bunt organism, but because they have been a medium through which the pathogene has become more thoroughly and widely distributed.

As a preliminary to the improvement of the hard red spring wheats at the University of Alberta all of the common varieties of spring wheat were tested for their reaction to various collections of bunt found in the province. The work was started in the spring of 1929, but owing to the drought, the infection percentage was too low and variable to be reliable. In the spring of 1930 conditions were more favorable for bunt infection, as is indicated by the high average infection percentage obtained on susceptible varieties.

In an earlier publication on breeding wheat for stem rust resistance (1) the writer has pointed out that, "crop improvement which is concerned with disease resistance must take into consideration: (a) the possible existence of physiologic forms of the pathogenic organism, (b) the need of a survey to determine the prevalence and distribution of the various forms, (c) the varietal reactions of the host to particular forms, (d) the reactions between host and parasite as definite genetic characters, and (e) the possibility of combining the resistance to all forms within a single desirable commercial variety." Investigations on the bunt problem have not advanced sufficiently to isolate the various natural divisions and organize a coherent plan of attack. In order to assure that any new wheat productions might be reasonably resistant to the forms prevalent in the province, the practice of using composite chlamydospore cultures as inoculum was employed. Inoculum was obtained from four general sources as follows: (a) infected heads of a number of varieties in the experimental plots at the University of Alberta, Edmonton, (b) material collected in the fields at various places in the province, (c) samples of smutty wheat from the Dominion Grain Inspection Office, Edmonton, and (d) samples of infected heads sent in by growers and other agencies.

It is recognized that the use of composite cultures of inoculum brings in a possible deviation in the infection percentages from that which might have been obtained if pure cultures were used. Heald (19) has demonstrated that spore load is an important factor in obtaining the maximum percentage of infection. A composite culture may be expected to possess varying inherent potentialities for attacking different varieties of wheat. It is obvious that it

would be practically impossible to have all the parasitic entities which go to make up the composite culture in the same proportions, especially when the virulence of the individual pathogenes and the reactions of the separate hosts are unknown. Even though the numbers of spores of the different cultures are approximately equal in a composite culture, there is still the likelihood of differences in viability of spores of the different cultures. Consequently it is to be expected that if there is a differential reaction of the varieties to the pathogenic forms in the composite inoculum, the test might not be comparable to the infection obtained by the use of individual cultures or forms. In using a composite culture it would not be difficult to conceive of a mixture of forms containing potentialities for infecting a particular variety in entirely different proportions from that of a second variety. In order to overcome the possibility of not obtaining the maximum infection, cultures, or forms, of bunt are carried individually through several generations on the varieties of wheat commonly grown in the prairie provinces of Canada. These cultures are then used as inoculum in separate tests on the standard varieties, parental material and the more promising hybrid selections.

In general, the results reported in this paper would seem to indicate that the use of composite cultures of chlamydospores for testing varieties of wheat for their reaction to bunt are sufficiently reliable for all practical purposes. The infection percentages obtained on different standard varieties when composite cultures were used as inoculum are in fairly close agreement with those obtained by other workers (19, 20, 21) and from field observations. In one of the physiologic-form experiments, evidence was obtained to the effect that the sum of the percentages of infection obtained through the use of separate cultures was approximately equal to the percentage obtained from a composite culture made from the same material. Hope wheat when inoculated with six separate cultures at 10° C. had infection percentages as follows: No. 1—7; No. 2—13; No. 3—0; No. 4—0; No. 5—0 and No. 6—0. When this wheat was inoculated with a composite culture of these forms the infection percentage was 22, or approximately the same as the sum of the percentages from the separate inoculations. Naturally such a relationship could hold only for varieties that are resistant since the sum of infection percentages of susceptible varieties would be well over 100.

The seed of 137 varieties and hybrid selections was heavily smutted with a composite collection of both *T. tritici* and *T. levis* before sowing. Approximately equal amounts of inoculum were applied to the seed of each variety, or hybrid, in order to make the spore load as uniform as possible. The dry chlamydospores and the seed were thoroughly shaken in the envelope until the surface of each kernel had a dark smutty appearance. The inoculated seed was sown in the field in rows one foot apart. The furrows were opened with a garden cultivator and the seed dropped and covered immediately in order to avoid drying-out of the soil.

The percentages of bunt were determined by making counts of the smutted heads and the total number of heads from which the infection percentage was

calculated. The plots were not replicated in the field in this preliminary experiment, but replicate plantings were made in the greenhouse, some of which were kept under controlled temperatures for the first two weeks after sowing.

In order to obtain some indication of the need for replication in bunt tests, a series of sixteen Marquis rows were sown as checks both in the field and greenhouse experiments. In the greenhouse the seed was sown in small wooden flats. The soil temperature in the greenhouse was too high when the seed was germinating, and consequently the infection percêntage was low. The greenhouse series of Marquis checks varied from 5 to 29% with an average of 17.5, and the field series from 38 to 88% with an average of 61.2. The data are presented graphically in Fig. 1.

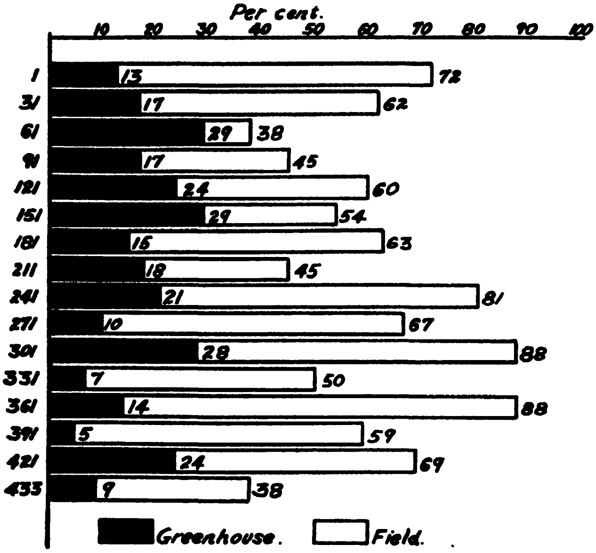


FIG. 1. *Bunt percentages in Marquis check plots grown in the greenhouse and in the field.*

These results demonstrate rather strikingly the need for replication in conducting experiments with bûnt. The variations are greater than was expected in view of the fact that the Marquis seed was elite stock, and that the samples of both seed and inoculum were handled in an identical manner. The seed sown in the greenhouse and that sown in the field came from the same prepared sample. The correlation coefficient between field and greenhouse infections is +0.1321 ± 0.139. This non-significant value is to be expected in view of the great degree of variability and the small number of tests.

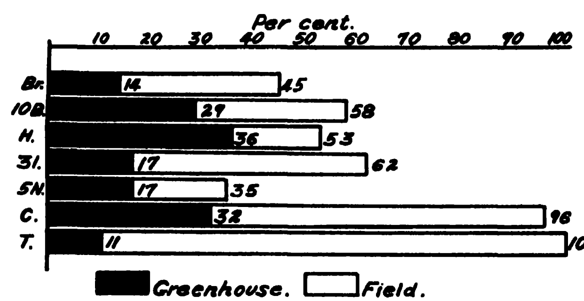


FIG. 2. *Bunt percentages on different strains of Marquis wheat in the greenhouse and in the field.*

Different stocks of an agronomic variety are usually considered as being practically equal in resistance to specific diseases. The soundness of this opinion is dependent to a considerable extent on the method of origin of the particular stock in question. Seed stock developed under the rigid rules and

regulations laid down by the Canadian Seed Growers' Association for the production of elite and registered seed might be expected to be uniform for most characteristics. Percentages of bunt infection were determined for seven strains of registered Marquis. The data are presented graphically in Fig. 2.

The results are similar to those obtained in the replicated plots from a single stock of Marquis. There is a range in infection from 11 to 36% in the greenhouse with an average of 22.3%, and 35 to 100% in the field with an average of 64.1%. One would not be justified in concluding that there is an inherent difference in the susceptibility of these strains of Marquis to bunt. The variability between individual plots is great. Average values from replicated plots of each strain are necessary before the inherent reactions to bunt of each strain could be determined.

The average infection percentage of the 149 varieties and selections was 15.3 in the greenhouse and 55.8 in the field. These figures approximate those of the Marquis checks in the same experiment. With the great increase in number of tests there is a significant correlation coefficient between the bunt percentages in the two tests of +0.4602 ± 0.044. The correlation surface showing the distribution of the bunt percentages for the 149 plots of wheat varieties and selections grown in the field and the greenhouse is illustrated in Table I.

TABLE I

CORRELATION SURFACE SHOWING THE DISTRIBUTION OF THE BUNT PERCENTAGES FOR 149 WHEAT VARIETIES AND SELECTIONS GROWN IN THE FIELD AND IN THE GREENHOUSE

Percentages of field infection	Percentages of greenhouse infection								
	0	5	15	25	35	45	55	65	Freq.
0	2								2
5	2								2
15	4	4	3	2					13
25		1		2					3
35	2	7	3	1	1				14
45	4	7	10	2					23
55	1	10	10	9	1				31
65	2	8	4	5	2				21
75	3	1	6	5	3				18
85	1		3	5	2	2	1		14
95		1	3		1	2		1	8
Freq.	21	39	42	31	10	4	1	1	149

$r_{xy} = +0.4602 \pm 0.044$

The correlation is high when one considers the great variation that was shown by the replicated check plots. The relationship between the results of the tests in the greenhouse and in the field indicates that these results are sufficiently reliable to be used as a preliminary indication of the reaction of the different varieties and selections to bunt. The infection percentages from the field tests of the commonly grown spring bread wheat varieties are given in Table II.

TABLE II

COMMON VARIETIES OF SPRING WHEAT SHOWING PERCENTAGE OF HEADS INFECTED WITH BUNT IN FIELD TESTS IN 1930

Variety	N.S.N.*	Percentages of bunted heads	Variety	N.S.N.*	Percentages of bunted heads
Red Bobs 222	I-0-18	92	Marquis	I-0-9	53†
Dicklow	I-30-4	88	Hard Federation	I-28-35	50
Reward	I-0-21	88	Reliance	I-29-4	49
Hard Federation	I-30-2	86	Renfrew	I-0-20	46
Kota	I-29-6	83	Huron	I-0-4	44
Red Fife	I-0-19	78	Marquis x Emmer		
Progress	I-29-7	77	(H_{55})	I-28-111	44
Ceres	I-25-1	71	Marquis x Emmer		
Preston	I-30-3	70	(H_{44})	I-28-112	36
Marquillo	I-29-8	65	Renfrew	I-0-20	33
Early Triumph	I-0-2	62	Kitchener	I-0-5	26
Producer	I-25-7	58	Ruby	S-22-42*	18
Supreme	I-0-23	55	Garnet	I-0-3	12

**N.S.N.* = *Nursery stock number.* †*Av. 16 plots.*

It will be noticed from a study of the data presented in Table II that the more recent productions, such as Kota, Ceres, Reward, Progress and Red Bobs 222, are all highly susceptible. Their percentages of bunt are 83, 71, 88, 77 and 92 respectively. The average of sixteen Marquis checks is 53%. These results indicate that the introduction and culture of new varieties less able to resist infection by the bunt organism, has contributed materially to the present increase in bunt on the farms in western Canada. Even though a new variety, such as Kota, meets with little favor from the grower, and is soon discarded, it is a most effective agent in disseminating the organism to farms and in regions where the disease had not previously been a particularly important problem.

The loose smut of wheat (*Ustilago tritici*) has received considerable attention during recent years. Its prominence can also be readily accounted for by the introduction of new susceptible varieties, such as Kota, Ceres and Reward. The seed of these new varieties distributed in western Canada was fairly heavily contaminated with loose smut. In preliminary variety tests Kota and Ceres have shown themselves to be highly susceptible to loose smut. Reward has often been referred to as a very susceptible variety. This can be accounted for by the fact that a portion of the first lots of seed that were distributed were contaminated. In varietal tests conducted by the writer where the wheats have been artificially inoculated, Reward has shown itself to be not nearly as susceptible as Kota and Ceres, and only slightly more so than Marquis.

Reward wheat is a very desirable type of hard red spring wheat for certain portions of western Canada because of its earliness and excellent milling and baking qualities (30). While considerable objection has been raised to it because of its susceptibility to loose smut, what is probably more important as a problem in the near future is its susceptibility to bunt. Fortunately the seed originally distributed seems to have been free from this disease. Bunt is

an accumulative disease. It may take several years before Reward is sufficiently contaminated to be called to the attention of most growers. Contact with bunt spores through machinery, bins, sacks and mechanical mixtures with other susceptible varieties on the farms will undoubtedly bring about in time a contamination of Reward. Its high degree of susceptibility as demonstrated by the tests reported in this paper and as indicated by a number of samples already received from growers, points to the necessity of regular seed treatment of this variety whether the stock is already contaminated or not.

Several of the newer varieties have brought into prominence another important factor in the spread of bunt, to which some reference has already been made. The results of a number of the investigators working on spring wheats (2, 26, 40) indicate that the durums, as a class, are less susceptible to *T. levis* and more susceptible to *T. tritici* than the hard red spring wheats. The reverse is true of the hard red spring wheats; their varieties have been less susceptible to *T. tritici* and more susceptible to *T. levis* than the durum wheats. Ceres, Progress and Kota are highly susceptible to both species of bunt and consequently may be important in the distribution of both of them. There is a great need for more exact and detailed knowledge regarding the reaction of new varieties previous to their distribution to the grower.

Several of the hybrid selections tested were free from infection in both the field and the greenhouse trials. These are to be tested further and if they continue to remain free from infection or are highly resistant they will be used as parental material in crosses.

Garnet wheat, while not immune, was the most resistant of the varieties commonly grown in western Canada. It had an infection of only 12%. These experimental results conform with observations in the field where it has been noticed that Garnet seldom contains bunt. Garnet was observed to be highly resistant to five of the six collections of bunt with which it was inoculated in the physiologic-form experiments (Fig. 9), and in the breeding studies where thirty replicated parental check rows had an average of 22% bunt when a composite inoculum was used (Table V). Physiologic form 2, a strain of *T. levis*, was much more virulent on Garnet than any of the six collections used in the physiologic-form experiment. Form 2 produced 56% bunt on Garnet. Physiologic form 1, a strain of *T. tritici*, produced only 7% under similar conditions.

One case of a naturally infested field of Garnet with 7% of the heads infected was observed at Egremont, Alberta, in 1930. The organism in this case was *T. tritici*. The echinulations on the spores were much more pronounced than on other collections of *T. tritici* made in the same year. A preliminary test was made with this collection in the laboratory and greenhouse, and Garnet was infected to the extent of 82 and 87% respectively. This collection is evidently much more virulent than form 1 on Garnet, and also on some of the other differential hosts. In the laboratory tests Hope had 71% of the plants infected and Mindum (a durum wheat), 86%.

Physiologic Forms

The discovery of physiologic forms of phytopathogenic organisms has contributed a wealth of knowledge to the understanding and control of plant disease by the production of resistant varieties. Knowledge concerning the number, prevalence and distribution of physiologic forms of any pathogenic organism is of such vital importance to the crop improver that he cannot afford to ignore or fail to give cognizance of the possibilities. To produce new varieties that are not resistant to all the forms of the pathogene which are present in the region in which the variety is to be grown is to acquiesce, at the best, to only temporary or partial success. The pathogenic forms present in those regions from which contamination is likely to arise, through such agencies as wind, water, etc., and the dissemination of the organism on the seed of the host, must also be considered. Plant quarantines are ineffective or only temporary in preventing the introduction of new inoculum where there is a natural exchange of materials, or where agencies which act as carriers in distributing the organism occur.

The existence of physiologic forms in the organisms causing powdery mildews and rusts, and in other plant pathogenes has been known for some time. Kniep (29), in 1919, was the first to show that there are physiologic forms in the smut fungi. The existence of physiologic forms in the smuts of cereals was first demonstrated in *U. hordei* (covered smut of barley) by Faris (12) in 1924, and by Reed (33) in *U. avenae* and *U. levis* (loose and covered smuts of oats). When studying the influence of various factors on the infection of wheat by *Tilletia tritici* and *T. levis* Faris (11) obtained some evidence of the existence of specialized races. His experiments were extended and the results were confirmed in 1927 (34) and 1928 (35). Miss Sampson (39) and Rodenhiser and Stakman (37) in 1927, have also presented some evidence of physiologic specialization in *Tilletia*. The latter working with the spring wheats Einkorn, Marquis and Kota as differential hosts compared the virulence of several collections of bunt from European countries with that of Minnesota collections.

In 1927, Stephens (20) at Moro, Oregon, showed that several wheats which had been practically smut free for several years, when inoculated artificially with local collections of bunt became heavily infected. Gaines (20) about the same time obtained a smut collection from Germany which was also very virulent on several of the same varieties infected by Stephens with his local bunt. These more virulent forms were shown to be strains of *T. levis* which species had not previously been prevalent in the state of Washington.

Roemer (20) working in Germany reciprocated with Gaines in the exchange of bunt collections and wheats. He likewise demonstrated the existence of several physiologic forms and suggested that each collection is probably a mixture of several pure lines.

Rodenhiser (38) in 1928 demonstrated again that several European collections of bunt and one from Egypt consisted of a number of physiologic forms of *T. levis*. He demonstrated also that several collections of *T. tritici* from

European countries, and one from New Zealand consisted of several physiologic forms. No evidence was presented regarding the existence of pathogenic forms from collections in the spring wheat area of the United States and Canada. Marquis, Kota, Mindum, Pentad and Einkorn, all spring wheats, were used as differential hosts to identify the physiologic forms.

Reichert (36) demonstrated that, in Palestine, forms of bunt were present which were especially virulent on the durum wheats.

Brentzel and Smith (2) in 1929, used nine varieties of durum wheat and twelve varieties of common wheat as differential hosts and found that the presence of virulent physiologic forms of *T. tritici* were responsible for the increase in prevalence of bunt on durum wheats. Common wheats, in general, were more susceptible to *T. levis* and the durum wheats more susceptible to *T. tritici*. The standard varieties of common wheat used as differential hosts were Hope, Preston, Marquillo, Reliance, Marquis, Ruby, Powers' Fife, Webster, Ceres, Progress, Haynes' Bluestem, Kota and Quality.

Holton (26) thought that in view of the increasing severity of bunt in the United States and the evidence contributed by Reichert, the presence of new and virulent strains of bunt was responsible for the outbreak of bunt on durum wheats in the spring wheat region. He used eight varieties of spring wheat as differential hosts, namely, Kota, Preston, Marquis, Marquillo, Hope, Mindum, Pentad and Vernal emmer. Three physiologic forms of bunt were found in the spring wheat region. One from Manitoba was especially virulent on Kota and of low virulence on the other seven varieties. A collection from North Dakota was especially virulent on the durum wheats, Mindum and Pentad. A third collection from Minnesota was distinguished by its higher relative virulence on emmer.

Heald and Gaines (24) have reported recently the results obtained from inoculating 22 wheats with seven separate collections of bunt. Their tests included several spring wheats among which were Marquis and Hope. Hope was immune from bunt and Marquis highly resistant when spring sown. Hope and Marquis were highly susceptible, however, when fall sown, indicating that climatic conditions and cultural practices are responsible for wide fluctuations in the degree of bunt infection. The trend in the states of Washington and Oregon over a period of years has been in the direction of increasing amounts of smut. This fact, combined with experimental evidence demonstrating that wheats which formerly were resistant are now susceptible, leads to the conclusion that new strains of bunt are responsible for some of the increase. Heald and Gaines call attention to the need for effective seed treatment to inhibit the spread and further development of these new and virulent forms.

Bressman (3) studied the behavior of 100 collections of bunt on 10 differential hosts and found six physiologic forms of *Tilletia levis* and four of *T. tritici*. Practically all of the varieties of wheat which had been classified formerly as resistant to bunt were susceptible to one or more of the physiologic forms. Hosar, a hybrid selection, was consistently resistant to all of the available collections of bunt fungi.

Holton (27) has recently confirmed his earlier work on the susceptibility of durum wheats to new forms of *T. tritici*. Evidence is also presented which shows that Vernal emmer and Marquis were more heavily bunted in 1930 than in 1929. It is concluded from these results that new and more virulent forms attacked these two varieties in 1930.

Most of the studies to date on the identification of physiologic forms of the bunt fungi have been made primarily with winter wheat varieties as differential hosts. The majority of these varieties are not sufficiently winter hardy to survive in the field in most years with the climatic conditions prevailing at Edmonton. When grown in the field from spring sowings, or in the greenhouse, the dormancy period of the winter wheats prolongs the experiment, or necessitates special treatment of the plants to avoid long dormancy periods. Consequently it would be very impracticable to use the usual winter wheat differential hosts for the identification of physiologic forms of bunt fungi in Alberta. With the rapidly increasing prevalence of bunt in spring wheats a number of investigators have selected spring wheats as differential hosts. Some of the varieties used by Rodenhiser and Stakman, Brentzel and Smith, and Holton were supplemented with a few others to use as differentials in these studies. The varieties are as follows: *T. vulgare*—Kota, Red Bobs, Bozosio, Progress, Preston, Marquis, Reliance, Garnet, Hope; *T. durum*—Pentad; *T. dicoccum*—White Spring Emmer; and *T. compactum*—Little Club.

The term "physiologic form" is applied in this paper to a purified chlamydospore collection of bunt capable of producing a definite set of reactions on a given group of wheat varieties. Flor (16) has demonstrated that *T. tritici* is heterothallic. Reduction division and segregation evidently take place before the fusion of sporidia to form the next sexual generation. Hybridization between monosporidial lines is necessary to bring about successful infection with subsequent chlamydospore production in the host. The sporidia which fuse and later produce chlamydospores may be similar except in sex. They may or may not have different parasitic capabilities, and for this reason the application of the term "physiologic form"—a term which implies a certain degree of homogeneity—to collections of chlamydospores might be questioned. It is to be expected that most collections would be mixtures of physiologic forms and capable of infecting a wide range of hosts. Few varieties make good differential hosts. Wheat varieties with varying capabilities for infection would act as screens to a certain extent in reducing the number of forms in a mixed collection. Dillon-Weston (10) has demonstrated that new forms may be isolated from the bunted wheat in England by using resistant hosts. The more resistant the host upon which a collection of chlamydospores is cultured the finer would be the screen through which the pathogenes would have to pass. A high degree of susceptibility would be more favorable to the carrying of a number of physiologic forms. Varieties more resistant, or selective, to parasitic invasion by particular forms would be better hosts upon which to maintain pure cultures. Using the same host for several successive generations would also be of considerable value. Briggs (6) has found a high degree of

stability and uniformity of reaction on particular hosts over a period of years and through numerous generations.

Considerable differences in virulence in chlamydospore collections have been demonstrated by a number of investigators. With consistently uniform reactions in a succession of generations, from most of the cultures it has seemed justifiable for all practical purposes to consider the different collections of chlamydospores varying in virulence as separate genetic entities. It is convenient and useful, therefore, to designate such collections of chlamydospores as physiologic forms.

Six collections of bunt were made on pure varieties of spring wheat in the experimental plots at the University of Alberta. The bunt on Red Bobs was *T. tritici* with a small contamination of *T. levis*, and the bunt on the other five varieties was *T. levis*. The seed of 12 varieties of wheat was inoculated separately with chlamydospores from each of the six collections of bunt. In addition one set of seed was inoculated with a composite culture made up of all the collections of bunt. A sixth set of seed of the 12 varieties was sown uninoculated as a check. Precautions were taken to avoid mixing of the inoculum in the preparation of the samples and in the sowing. Hands and equipment were thoroughly washed and disinfected between handlings of the different lots of material.

Faris (11) and Reed (35) report that low temperatures approximating 10° C. were the most favorable for obtaining high infection on susceptible varieties. As a preliminary to these studies four series of wheat were inoculated with a composite culture of bunt in order to determine the condition that would be most favorable for infection. Two series were sown in wooden flats filled with soil and then placed in freezing chambers and the seed germinated at controlled temperatures, one series at 5° C. and another at 10° C. The third series was sown in the field on April 23, when the soil temperature at seeding depth was approximately 10° C. The fourth series was sown in the field on May 15, when the soil temperature was approximately 15° C.

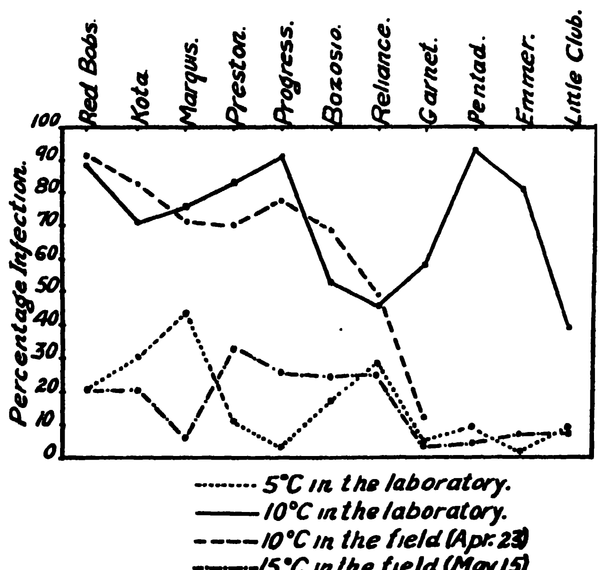


FIG. 3. *Bunt percentages on eleven varieties of wheat when the inoculated seed was germinated at different temperatures in the laboratory and field.*

Eleven varieties of wheat were used in each series except the early sown field series, which lacked three of the varieties. The average per cent infection for the eight varieties of wheat common to all four series was 65.4 when sown in the field on April 23 with a soil temperature of approximately 10° C. and only 19.6% when sown on May 15 with a soil temperature of approximately 15° C. In the laboratory the series germinated at 10° C. had an average per cent infection of 70.8 and only 20.0% when germinated at 5° C. The results obtained in this experiment are shown graphically in Fig. 3.

These results demonstrate and emphasize the importance of germinating seed inoculated with bunt spores at favorable temperatures for infection. A temperature of approximately 10° C. appears to be the most favorable for obtaining high infection on susceptible varieties. In these experiments the soil moisture was approximately 20-25%.

Six sets of the 12 differential hosts were inoculated with bunt collections from 1—Red Bobs, 2—Kota × Marquis, 3—Reliance, 4—Bozosio, 5—Ruskier, and 6—Saaminkil. The seed was sown in the field 1½ in. deep when the soil temperature was approximately 15° C. A seventh set of differential hosts remained uninoculated to serve as checks. The sets inoculated with the collections from Red Bobs, Kota × Marquis and Reliance were triplicated by sowing two additional sets in the laboratory and germinating the seed at 5° and 10° C. Uninoculated seed of each variety was sown again to serve as checks. Without exception the checks in all the experiments remained free from infection. The results are summarized in Table III.

TABLE III

PERCENTAGE OF BUNTED PLANTS IN 12 VARIETIES OF WHEAT INOCULATED ARTIFICIALLY WITH SIX COLLECTIONS OF *Tilletia* AND GERMINATED AT 10° AND 15° C.

Variety or differential host	Source of inoculum and percentage of bunted plants										
	1 Red Bobs		2 Kota × Marquis		3 Reliance		4 Bozosio	5 Ruskier	6 Saaminkil	Average at	
	10°C.	15°C.	10°C.	15°C.	10°C.	15°C.	15°C.	15°C.	15°C.	10°C.	15°C.
Kota	67	14	90	32	90	5	19	31	50	82	25
Progress	81	0	86	43	73	0	6	0	22	80	12
Bozosio	76	11	68	3	51	4	19	24	47	65	18
Red Bobs	60	41	63	61	41	0	8	10	46	55	28
Preston	56	15	35	28	59	0	0	50	31	50	21
Reliance	52	8	67	3	32	0	7	22	9	50	8
Marquis	65	0	23	12	40	4	29	40	5	43	15
Garnet	7	1	56	3	22	0	0	6	2	28	2
Hope	7	0	13	0	0	1	0	0	0	7	0
Little Club	38	15	62	0	32	0	67	0	0	44	14
Pentad	69	0	54	15	64	11	16	48	20	62	18
Emmer	50	0	69	20	70	5	7	12	13	63	10
Average	52.3	8.8	57.2	18.3	47.8	2.5	14.8	20.2	20.4	52.4	14.2

The infection percentages for collections 1 to 3 in Table III were taken from the series germinated in the laboratory at 10° C. The higher percentages of infection obtained in that experiment make it easier to differentiate between the reactions of the different collections and also make the comparisons more reliable. Collections 4 to 6, Table III, were studied in the field only where the soil temperature was approximately 15° C. at the time of seeding. The percentages of infection are not as high as were obtained with collections 1 to 3 but their reactions are markedly different on the various hosts.

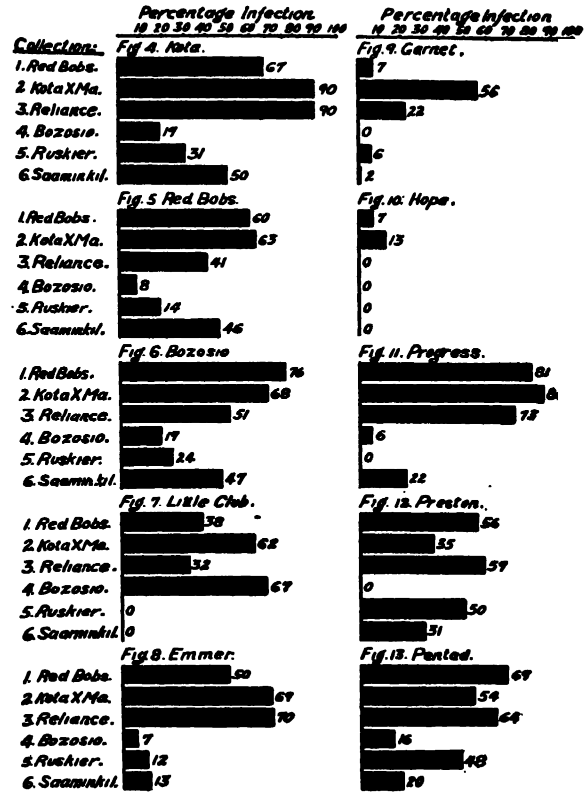


FIG. 4–13. *Bunt percentages on ten varieties of wheat when inoculated separately with six collections of bunt.*

The six collections of bunt showed approximately the same reactions on Kota, Red Bobs and Bozosio (Fig. 4-6). On the balance of the differential hosts shown in Fig. 7 to 12, the reactions of the six collections are sufficiently contrasted to be able to classify the collections as separate entities, or physiologic forms. In order to facilitate a detailed examination of the data the percentages of infection are classified as follows and summarized in Table IV.

0– 6% infection = highly resistant = HR
7– 15% infection = resistant = R
16– 30% infection = moderately resistant = MR
31– 45% infection = moderately susceptible = MS
46– 60% infection = susceptible = S
61–100% infection = highly susceptible = HS

The higher the degree of susceptibility the greater is the fluctuation in the percentages obtained, consequently it seemed reasonable to require greater differences the higher the susceptibility. The difference in infection percentage is 7 between the first two classes, 15 between the next three and 40 between the last two classes.

TABLE IV

THE REACTION OF TWELVE WHEAT VARIETIES INOCULATED WITH SIX COLLECTIONS OF *Tilletia*

Variety	Source of inoculum					
	1 Red Bobs	2 Kota × Marquis	3 Reliance	4 Bozosio	5 Ruskier	6 Saaminkil
Kota	HS	HS	HS	MR	MS	S
Red Bobs	S	HS	MS	R	MR	S
Bozosio	HS	HS	S	MR	MR	S
Progress	HS	HS	HS	HR	HR	MR
Little Club	MS	HS	MS	HS	HR	HR
Pentad	HS	S	HS	MR	S	MR
Emmer	S	HS	HS	R	R	R
Preston	S	MS	S	HR	S	MS
Marquis	HS	MR	MS	MR	MS	HR
Reliance	S	HS	MS	R	MR	R
Garnet	HR	S	R	HR	HR	HR
Hope	R	R	HR	HR	HR	HR

Little Club is highly susceptible to forms 2 and 4, moderately susceptible to forms 1 and 3, and highly resistant to forms 5 and 6 (Fig. 7). Emmer is highly susceptible to forms 2 and 3, susceptible to form 1, and resistant to forms 4, 5 and 6 (Fig. 8). Garnet is susceptible to form 2, resistant to form 3, and highly resistant to forms 1, 4, 5 and 6 (Fig. 9). Hope is highly resistant to forms 3, 4, 5 and 6, and resistant to forms 1 and 2 (Fig. 10). Progress is highly susceptible to forms 1, 2 and 3, highly resistant to forms 4 and 5, and moderately resistant to form 6 (Fig. 11). Preston is susceptible to forms 1, 3 and 5, moderately susceptible to forms 2 and 6, and highly resistant to form 4 (Fig. 12). Pentad is highly susceptible to forms 1 and 3, susceptible to forms 2 and 5, and moderately resistant to forms 4 and 6 (Fig. 13).

Forms 1 and 3 are the only collections the reactions of which do not differ on one or more of the host varieties by at least two classes. Since form 1 is *T. tritici* and form 3 is *T. levis* they can still be considered as different forms on the basis of morphology. The similarity in reaction between these two collections may be due in part to the fact that form 1 showed some *T. levis* spores which in all probability were not unlike those of form 3 in pathogenicity.

Differences in infection percentages at varying temperatures is another basis upon which some of the physiologic forms might be differentiated. From the data presented in Table III it will be seen that form 3 is practically as virulent at 10° C. as forms 1 and 2, especially on the two susceptible varieties Kota and Red Bobs. At 15° C. forms 1 and 2 are still fairly virulent, while form 3

appears to be very non-virulent. Form 3 from Reliance appears to be only weakly parasitic at 15° C.

It is interesting to note that certain of the varieties are more susceptible at one temperature than at another. Progress was equally susceptible to forms 1 and 2 at 10° C., the infection percentages being 81 and 86 respectively. At 15° C. the infection percentages were 0 and 43 respectively for forms 1 and 2. Red Bobs reacted approximately the same at both temperatures with both bunt forms.

Several investigators have reported that in general common wheats are more susceptible to *T. levis*, and the durum wheats are susceptible to *T. tritici*. In these experiments (Fig. 13) 69% of the Pentad (durum) plants were infected with form 1 (*T. tritici*). This same form was also very virulent in most of the common wheats. Forms 2, 3 and 5 (*T. levis*) infected Pentad heavily but not quite to the same degree as form 1. The infection percentages were 54, 64 and 48 respectively. It would appear from these results that in general the common wheats are more frequently infected with *T. levis* but that forms of this species are not necessarily less virulent on the durum wheats than forms of *T. tritici*.

The existence of several physiologic forms differing in their pathogenicity emphasizes the need for a consideration of this phase in the problem of breeding for resistance to bunt. The mere enumeration or classification of numerous physiologic forms is of no particular importance or value to the plant breeder. However, exact knowledge regarding the genetics of bunt resistance can be obtained only from studies with known physiologic forms and pure hosts. It is important therefore to use definite forms whose capacity for infecting parental varieties is fully known. The material just presented is preliminary work leading to an attack upon the wheat bunt problem.

Breeding

Since the breeding studies reported herein are only of a preliminary nature no extensive review of the literature on this phase of the problem seems necessary at this time. A very complete and extensive review was given by Gaines (19) in 1923 and has been well supplemented recently by Briggs (4) in 1926, and again in 1930 (6).

Farrer (13, 14, 15) in Australia as early as 1901 began work on the development of bunt resistant varieties by hybridization. His plan was to subject F_2 and F_3 plants to a heavy attack by bunt and then isolate the resistant selections. The work of Farrer was carried on by his assistants after his death in 1906. This work resulted in the production of two resistant varieties now known as Florence and Genoa.

Little work was done on the breeding of wheats resistant to bunt outside of Australia until 1915, when Gaines (18) began his studies in the United States. In addition to the production of resistant varieties Gaines made a considerable contribution to the knowledge on the genetics of bunt resistance. He crossed varieties possessing varying degrees of resistance to bunt. There were accumulative effects in some of the hybrids and he concluded that multiple genetic factors governed the reaction of the host to the pathogene.

With the discovery and demonstration of the existence of several physiologic forms in the bunt organism additional complications have arisen in the study of the genetics of bunt reaction. This condition necessitates the use of known physiologic forms of the causal organisms in genetic studies. Briggs (4, 5, 6, 7, 8) working in California has made considerable progress in this direction. He has demonstrated the existence of at least two independently inherited dominant factors for resistance. The isolation of the different factors for resistance in the commonly grown varieties of wheat, especially on those varieties to be used as parents in crosses, will be of considerable assistance in the production of varieties resistant to all known pathogenic forms of the causal organism. While detailed knowledge regarding the mode of inheritance of reaction to bunt will be of great help in planning an intelligent attack on the problem, still its lack should not inhibit the initiation of a breeding program in which the hybrid material is inoculated with composite collections of spores from regions in which the improved variety is to be grown.

The breeding studies were initiated for the purpose of improving the commonly grown wheat varieties in their abilities to resist attacks by pathogenic organisms; to withstand unfavorable climatic conditions during the normal growing season, and to improve the quality of the grain, especially when grown on the marginal wooded soils that are lacking in nitrogen. The various hybrid lines and parental varieties are exposed in the segregating generations to as many of these factors as the limited quantity of seed will permit. This is done by making replicate plantings in the different environments and in artificially produced disease epidemics.

When breeding for bunt resistance there are two good reasons for not infecting the F_1 and F_2 plants. The first is that if the plants are susceptible and infection is successful the plants fail to produce seed and are lost for study in later generations. The second is that some susceptible plants nearly always escape infection and their true reaction either is not determined, or their progeny will have to be tested in the succeeding generation. It is more feasible to classify the F_2 plants on the basis of the infection percentages of the F_3 lines. Naturally a limited amount of the crossed and F_1 seed could be inoculated when an indication of the genetic factors operating in the earlier generations is desired. Unless conditions are particularly favorable for infection such that practically all susceptible plants become infected, the ratios of susceptible and resistant plants would not be as reliable as those obtained from F_3 lines.

In these studies the seed produced by the F_2 plants was thoroughly inoculated with chlamydospores from composite collections similar to those used in the varietal test. The seed was sown by hand in five-foot rows one foot apart in 50, 75 or 100 seed plots. The seeding was done when the soil temperature was approximately 10° C. Parental check rows treated similarly to the hybrid material were sown every 30 plots. The field had been summerfallowed in the previous season, so that it would be in good tilth with sufficient moisture for prompt germination, and free from volunteer wheat. At harvest time the

plants were pulled and separated into two classes, bunted and bunt-free. A plant was classified as bunted if it showed any infection whatever. The percentage infection was then calculated from the data thus recorded.

Nine varieties of common wheat were used as parents in this breeding study. Two varieties, Garnet and Reward, were used primarily because of their early maturity and general adaptability to northern regions. Both varieties are able to produce grain of good appearance on poor soils. Reward is superior in baking quality to Garnet and most other varieties commonly grown in western Canada. H_{35} and H_{44} are hybrid selections from a Marquis × Emmer cross made by McFadden (31) in the United States. They are resistant to stem rust and have shown some resistance to bunt at other places, but are not of especially good quality or yielding ability.

The other five parental varieties are pure line selections from crosses between Marquillo (Marquis × Iumillo) and several Marquis × Kanred hybrid selections. Because of the manner in which they originated they are usually referred to as Double Crosses (D.C.). They rank high in yield and quality and have shown considerable resistance to foot-rots and stem and leaf rusts. One of them, I-28-46, has also shown moderate resistance to bunt. These hybrids originated at St. Paul, Minnesota, in the co-operative wheat breeding projects between the Division of Cereal Crops and Diseases of the United States Department of Agriculture, and the Sections of Plant Breeding and Plant Pathology of the University of Minnesota, with which the writer was formerly connected.

The percentages of bunt infection of the parental varieties and the variability in the different check rows are shown in Table V.

TABLE V

PERCENTAGE OF BUNTED PLANTS IN REPLICATED PLOTS OF NINE VARIETIES OF WHEAT INOCULATED ARTIFICIALLY WITH A COMPOSITE CULTURE OF *Tilletia*

Variety	Total number of plots	Number of plots for different infection percentages										
		0	5	15	25	35	45	55	65	75	85	Average
Garnet	30	1	6	8	9	3	2	—	1	—	—	22
D.C. I-28-46	14	1	1	2	3	7	—	—	—	—	—	27
H_{35}	19	—	2	4	5	5	—	3	—	—	—	28
H_{44}	13	—	—	—	4	2	6	1	—	—	—	39
D.C. I-28-125	11	—	—	1	1	2	2	4	1	—	—	43
D.C. I-28-124	23	—	—	—	1	5	6	7	2	2	—	49
D.C. I-28-62	14	—	—	1	—	3	1	5	4	—	—	51
D.C. I-28-60	13	—	—	—	—	2	4	1	2	2	2	59
Reward	13	—	—	1	—	1	1	3	2	4	1	63

Three varieties, namely, Garnet, D.C. I-28-46 and H_{35} are moderately resistant to bunt and the balance are moderately susceptible or susceptible. There is a great deal of variability in the infection percentages as is illustrated in the frequency distribution in Table V. While these varieties are pure lines for all ordinary morphological characters there was no absolute certainty that they were pure lines as far as bunt reaction is concerned. When the seed

was prepared for the parental check plots several check plots of each variety were sown with seed from the same individual plant. The variability within these replicated plots sown with seed from individual plants could then be compared with that of bulk seed of pure lines. The percentages of bunt infection in replicated plots from individual plants are shown in Table VI.

TABLE VI

PERCENTAGE OF BUNTED PLANTS IN REPLICATED PLOTS SOWN WITH SEED FROM INDIVIDUAL PLANTS OF NINE VARIETIES OF WHEAT INOCULATED WITH A COMPOSITE CULTURE OF *Tilletia*

Variety	Plant number	No. of plots	Number of plots for different infection percentages										
			0	5	15	25	35	45	55	65	75	85	Average
Garnet	193	6	—	1	1	2	1	—	—	1	—	—	28
	199	5	1	2	—	1	—	1	—	—	—	—	18
	258	7	—	2	3	1	—	1	—	—	—	—	19
	249	4	—	—	1	2	1	—	—	—	—	—	25
	276	4	—	—	2	2	—	—	—	—	—	—	22
	284	2	1	—	—	—	1	—	—	—	—	—	20
D.C. I-28-46	238	4	—	1	—	1	2	—	—	—	—	—	26
	257	8	1	—	1	1	5	—	—	—	—	—	30
H_{35}	181	5	—	1	1	2	1	—	—	—	—	—	20
	184	6	—	1	—	2	2	—	1	—	—	—	29
	187	5	—	—	1	1	1	—	2	—	—	—	37
	190	2	—	—	2	—	—	—	—	—	—	—	18
H_{44}	155	6	—	—	—	1	1	3	1	—	—	—	43
	158	7	—	—	—	3	1	3	—	—	—	—	36
D.C. I-28-125	172	8	—	—	1	1	1	—	4	1	—	—	44
	177	3	—	—	—	—	1	2	—	—	—	—	42
D.C. I-28-124	154	7	—	—	—	—	2	1	1	1	2	—	55
	157	7	—	—	—	1	2	1	3	—	—	—	44
	186	4	—	—	—	—	1	1	1	1	—	—	44
	189	2	—	—	—	—	—	1	1	—	—	—	50
D.C. I-28-62	192	3	—	—	—	—	—	—	2	1	—	—	58
	198	4	—	—	1	—	—	—	1	2	—	—	50
	219	7	—	—	—	—	3	1	2	1	—	—	48
D.C. I-28-60	275	10	—	—	—	—	1	3	1	2	1	2	61
Reward	220	7	—	—	1	—	1	1	—	1	2	1	61
	239	2	—	—	—	—	—	—	1	1	—	—	61
	324	3	—	—	—	—	—	—	2	—	1	—	64

From a study of the data presented in Tables V and VI it is evident that in general the variability in bunt infection on different plots sown with seed from the same plant is as great as the variability in infection from seed of different plants of the same variety. The variations in infection then can readily be attributed to differences in environmental conditions and the chance of infection with the limited number of individuals used in plant rows, rather than to any inherent differences in the individual plants within each variety.

Large F_3 populations were tested for their reactions to bunt and the average infection percentage of each line was used as an index of resistance or susceptibility. Approximately 150,000 plants were grown and studied in the nine different crosses. The data have been summarized and the results are presented in Table VII.

TABLE VII

PERCENTAGE OF BUNTED PLANTS IN PARENT VARIETIES AND F_3 LINES WHEN INOCULATED ARTIFICIALLY WITH A COMPOSITE CULTURE OF *Tilletia*

Variety or cross	Total number of plots or F_3 lines	Number of plots for different infection percentages											
		0	5	15	25	35	45	55	65	75	85	95	Average
Reward	7	—	—	1	—	1	1	—	1	2	1	—	61
D.C. I-28-62	7	—	—	—	—	3	1	2	1	—	—	—	48
Reward × I-28-62	91	—	—	2	3	9	14	21	19	20	3	—	58
Reward	3	—	—	—	—	—	—	2	—	1	—	—	64
D.C. I-28-60	2	—	—	—	—	1	1	—	—	—	—	—	42
Reward × I-28-60	71	—	—	2	1	8	12	14	14	16	4	—	58
D.C. I-28-124	14	—	—	—	1	4	2	4	1	2	—	—	49
H_{44}	13	—	—	—	4	2	6	1	—	—	—	—	39
I-28-124 × H_{44}	260	—	3	9	34	45	62	48	34	14	8	3	48
Reward	3	—	—	—	—	—	—	1	1	1	—	—	67
D.C. I-28-46	5	—	1	1	2	1	—	—	—	—	—	—	22
Reward × I-28-46	97	—	2	2	2	5	21	23	24	12	3	3	46
D.C. I-28-60	11	—	—	—	—	1	3	1	2	2	2	—	62
Garnet	11	—	1	3	5	2	—	—	—	—	—	—	22
I-28-60 × Garnet	264	8	31	68	46	39	40	21	6	4	—	1	30
D.C. I-28-62	7	—	—	1	—	—	—	3	3	—	—	—	53
Garnet	11	1	3	1	3	1	1	—	1	—	—	—	24
I-28-62 × Garnet	200	3	9	20	35	43	45	23	18	4	—	—	38
D.C. I-28-124	9	—	—	—	—	1	4	3	1	—	—	—	50
H_{35}	9	—	—	4	1	2	—	2	—	—	—	—	30
I-28-124 × H_{35}	140	—	10	10	24	26	31	22	10	5	2	—	39
D.C. I-28-125	11	—	—	1	1	2	2	4	1	—	—	—	43
H_{35}	10	—	2	—	4	3	—	1	—	—	—	—	26
I-28-125 × H_{35}	277	1	20	40	62	56	53	25	26	4	—	—	33
D.C. I-28-46	9	1	—	1	1	6	—	—	—	—	—	—	29
Garnet	8	—	2	4	1	—	1	—	—	—	—	—	19
I-28-46 × Garnet	136	4	5	24	25	28	22	15	8	4	1	—	35

From a study of the distributions in Table VII it will be noticed that in every cross a number of F_3 lines transgressed beyond the range shown by both parents. This may be due in part to the greater number of individual hybrid plots than parental plots, but it is rather indicative also that several genetic factors govern the reaction to bunt and that numerous recombinations have been produced.

For convenience the parent and hybrid populations may be grouped into five major categories on the basis of reaction to bunt. The first two crosses in Table VII are susceptible × susceptible; the third cross, moderately susceptible × moderately susceptible; the fourth, fifth and sixth crosses, susceptible × moderately resistant; the seventh and eighth crosses, moderately susceptible × moderately resistant; and the ninth cross, moderately resistant × moderately resistant. In every cross but one the average infection percentage of the F_3 hybrid lines was intermediate between those of the two parents. In the ninth cross, between Garnet and D.C. I-28-46, both moderately resistant, the average infection percentages are 19 and 29 respectively for the two parents and 35 for the 163 F_3 hybrid lines. In this cross there is a very decided transgressive segregation toward a greater susceptibility.

There is a great similarity between the distributions of F_3 lines originating from different F_2 populations within the same cross. There were three F_2 families in the cross between D.C. I-28-125 and H_{35} from which 277 F_3 lines were grown, and two F_3 families in the cross between D.C. I-28-124 and H_{44} from which 260 F_3 lines were grown. The distributions of the F_3 lines for each F_2 population in these two crosses are shown in detail in Table VIII. In general this same degree of similarity between different F_2 families was present in all of the crosses studied. They are so similar that one would be justified in concluding that the parental material must have been homozygous for the genetic factors governing the reaction to bunt.

TABLE VIII

DISTRIBUTION OF PERCENTAGE BUNTED PLANTS IN F_3 LINES ORIGINATING FROM SEPARATE F_2 FAMILIES WHEN INOCULATED ARTIFICIALLY WITH A COMPOSITE CULTURE OF *Tilletia*

F_2 family	Total no. of F_3 lines	Number of F_3 lines for different infection percentages											
		0	5	15	25	35	45	55	65	75	85	95	Average
D.C. I-28-125 × H_{35}, No. 174	117	—	7	17	17	27	25	12	11	1	—	—	37
D.C. I-28-125 × H_{35}, No. 175	35	—	2	7	10	8	5	1	1	1	—	—	29
D.C. I-28-125 × H_{35}, No. 176	125	1	11	16	35	21	23	12	4	2	—	—	33
D.C. I-28-124 × H_{44}, No. 156	138	—	2	4	20	17	32	29	20	8	4	2	48
D.C. I-28-124 × H_{44}, No. 159	122	—	1	5	14	28	30	19	14	6	4	1	47

Discussion

Crop improvement programs are too often limited to the consideration of one or two characters which at the moment are of major importance. When a characteristic that may assume the role of a limiting factor in production has been disregarded in a breeding project, the improved variety is likely at any time to be a failure as far as the grower is concerned. This applies particularly to the problem of breeding for resistance to the many destructive diseases common to our field crops. It also applies to such important agronomic and commercial characters in cereals as stiffness of straw, time of maturity, shattering, milling and baking quality, etc. The need for superiority in these

characters is more generally appreciated and sought after in general agronomic breeding programs than in disease reactions. Ceres wheat is a variety that was produced and distributed for its resistance to black stem rust. Later it was found to be highly susceptible to leaf rust, bunt, loose smut, scab and foot-rot. Its predecessor, Kota, was equally undesirable, but was also of inferior milling and baking quality. One of the more recently distributed new varieties, Reward, is very desirable in fulfilling the needs of short season regions. It is also desirable in most other agronomic and commercial characteristics. It is equal, if not superior in baking quality, to Marquis, our present standard variety. It is, unfortunately, very susceptible to bunt. In the tests reported herein 88% of the plants were affected with bunt. Owing to the fact that it is a new variety not many complaints have, as yet, been received from the growers. Bunt is an accumulative disease, however, and in time this weakness will become evident in the field, unless efficient and consistent seed treatment is practised.

Garnet is another early variety which was distributed to fill the same needs as that supplied by Reward. Instead of being equal to Marquis it is inferior, especially when grown on poor or marginal soils, although the appearance may be good (30). It is, however, fairly resistant to bunt. In the same tests with Reward it had only 12% of the plants infected. It was the most resistant of all of the commonly grown varieties tested.

At this stage in the development of crop improvement work it is hardly to be expected that the new productions will be perfect in all respects. Some varieties are highly desirable, even though they possess one or two weaknesses. The important thing is to have some knowledge regarding the reaction of new varieties to the more important limiting factors before they are distributed. It is not fair to the grower to be advised to grow a new variety and then have him proceed to discover its disadvantages or weaknesses. If the information were available first the new variety could be distributed with instructions regarding any necessary precautions for its proper handling. Reward wheat is highly desirable in certain sections of the country, but it should be treated for bunt regularly if the seed is infected or in danger of becoming contaminated.

Once a variety is developed resistant to any particular disease, every precaution should be taken to avoid possible contamination by introducing new physiologic forms from other regions. Heald and Gaines (24) and Holton (27) have suggested that for this reason it would seem important to continue the treatment of all seed, especially new varieties of wheat for bunt. This procedure would lessen to some extent the value and importance of resistant varieties to the grower, since the cost of seed treatment, when considered on the basis of 20 to 25 million acres annually in Canada, is an important item. Naturally the more important advantages are the reduction of losses in the field due to lowering of yields and of grade. The possible necessity of treating seed of varieties that are resistant to the pathogenes in the region where they are grown, in order to avoid contamination by new virulent forms from the outside which are likely to be introduced, emphasizes the need for

precaution in introducing cultures of organisms from foreign countries, and the need for strict plant pest quarantine regulations.

Most varieties of field crops are soon contaminated with admixtures of other varieties. Seed treatment would prevent the infection of any susceptible admixtures, even though the variety itself were highly resistant or immune. An infection of 1% will often cover the threshed grain with sufficient smut to cause the whole crop to grade smutty (23). Purity of variety is one of the best precautions against having a normally resistant variety grade smutty.

The origin of physiologic forms has an important relation to the crop improvement program since the stability and permanence of the resistance bred into a new variety will, to a large extent, determine its usefulness to the grower. New forms may be introduced into the hard red spring wheat region from outside regions, or they may arise as a result of mutation or hybridization. New varieties introduced to control other diseases, or for some particular agronomic characteristic, may be heavily attacked by forms already present but unnoticed because of their low virulence on the older and more resistant varieties. The origin or increase of new physiologic forms by any or all of these methods has been demonstrated in a number of phytopathogenic organisms. There is no good reason for not believing that new forms of *T. tritici* and *T. levis* can originate through these same processes.

Flor (16) has demonstrated recently that monosporidial cultures inoculated into wheat seedlings produced no infection. Heterothallism was indicated when infection was obtained only from mixtures of two monosporidial cultures. These results indicate that, unless there are some homothallic forms as yet undiscovered, hybridization is requisite to successful infection. A resistant variety then must be resistant to all the potential combinations brought about by the natural segregation and recombination of factors for pathogenicity in the parasites. The importance of the range of virulence of any particular culture, or combination of cultures, and their host relationships comes into view.

New physiologic forms of *T. tritici* and *T. levis* have made their appearance in the hard red spring wheat regions of western Canada and the United States. The number and variability of the forms discovered will depend to a considerable extent upon the intensity and nature of the research program on the bunt problem. With an increase in the number of varieties used as differential hosts and in the number of collections of bunt it is conceivable that an unlimited number of forms could be demonstrated. Apart from demonstrating that great variability does exist in the organism as well as the host the mere demonstration of numerous forms may be of no particular value to the plant breeder. It is important, however, that exact knowledge be obtained as to the range in virulence of the pathogenes and the genetic factors governing the host reactions to them. Information regarding the prevalence and distribution of physiologic forms of bunt in the region for which the crop improvement program is designed is a necessity and basic to a well rounded out plant breeding program for resistance to bunt.

There is no absolute assurance that varieties of wheat which have been developed for resistance to bunt in any particular region will remain resistant indefinitely. New or undiscovered forms may arise within the region, or they may be introduced from the outside. This may be true of practically all plant pathogenes, but it has not prevented the production and successful use of numerous varieties of resistant crop plants in reducing losses from destructive diseases.

The presence of strains of pathogenic organisms with varying potentialities as parasites demonstrates that for unknown periods in the past variability has been brought about by those agencies responsible for the usual evolutionary processes. The opinion is sometimes expressed, or inferred, probably unintentionally, that the discovery of these forms suddenly accelerates their production to such an extent that breeding plants for resistance to disease is a hopeless task. The discovery of physiologic forms of a pathogenic organism does not necessarily intensify, or change, the status of the plant breeding problem. Such knowledge should help in the solution of the problem. The complexity of physiologic specialization and its relation to breeding for disease resistance appears to be an enigma for those who fail to appreciate the usefulness of such knowledge in the production of resistant varieties.

Summary

1. A study has been made of the reaction of numerous spring wheat varieties and hybrid selections to several chlamydospore collections of *Tilletia tritici* and *T. levis*, the causal organisms of bunt in wheat.

2. An increase in the amount of bunt in western Canada can be accounted for in part by the use of certain varieties that are more susceptible to this disease than some of those grown formerly.

3. The spring wheats show all gradations in reaction to bunt varying from apparent immunity to high susceptibility.

4. Composite cultures of chlamydospores grown on a number of wheat varieties were used as inoculum when testing varieties and hybrids for their reaction to bunt. The results obtained indicate that such tests are sufficiently reliable to be of great value in determining the reaction of a variety to bunt.

5. Replication of tests is necessary for an accurate determination of varietal reaction.

6. Durum wheats, as a class, have been reported as less susceptible to *T. levis* and more susceptible to *T. tritici* than the hard red spring wheats. The reverse is the case for the hard red spring wheats. Several new varieties such as Kota, Ceres and Progress are highly susceptible to both species of bunt fungi and consequently may be important in the distribution of both of them.

7. *T. levis* is more frequently found on the common wheats than *T. tritici*, but all forms are not necessarily less virulent on the durum wheats than *T. tritici*.

8. The varieties of spring wheat grown in the prairie provinces may be classified into three groups. First, varieties such as Kota, Ceres, Progress, Red Fife, Red Bobs, Preston and Reward that are highly susceptible. Second,

varieties such as Marquis, Renfrew, Reliance, Huron and Kitchener that are intermediate in susceptibility. Third, varieties such as Garnet and Ruby that are fairly resistant, but not sufficiently so as to make seed treatment unnecessary.

9. Commercial immune varieties of spring wheat are not yet available, but Garnet is the most resistant of any commonly grown.

10. The highest per cent infection was obtained when the inoculated seed was sown in soil with a temperature of approximately 10° C.

11. Control of bunt has been complicated by the existence of physiologic forms of *T. tritici* and *T. levis*. Certain varieties of spring wheats are resistant to some forms and susceptible to others.

12. One physiologic form of *T. tritici* and five of *T. levis* were obtained from six collections of bunt. A considerable number of forms could probably be distinguished with numerous collections and the proper differential hosts.

13. When subject to different temperatures, some physiologic forms appeared to respond differently in infection capabilities.

14. The varieties of wheat used as differential hosts are as follows: *T. vulgare*—Kota, Red Bobs, Bozosio, Progress, Preston, Marquis, Reliance, Garnet and Hope; *T. durum*—Pentad; *T. dicoccum*—White Spring Emmer; and *T. compactum*—Little Club.

15. The isolation and study of relatively pure forms of the organism will be necessary for a study of the genetic factors in the host governing the reaction to this disease.

16. Collections of chlamydospores cultured on pure host material for two or three generations should for all practical purposes be sufficiently homogeneous to be considered a physiologic form.

17. The term "physiologic form" is applied in this paper to a purified chlamydospore collection of bunt capable of producing a definite set of reactions on a given group of wheat varieties.

18. The inheritance of resistance to bunt was studied in the F_3 of crosses of (a) susceptible × susceptible; (b) moderately susceptible × moderately susceptible; (c) susceptible × moderately resistant; (d) moderately susceptible × moderately resistant; and (e) moderately resistant × moderately resistant, varieties of spring wheat.

19. The average infection percentage of the F_3 lines was intermediate between that of the two parents in eight of the nine crosses. In the latter case the average infection percentage of the F_3 lines was greater than that of either parent.

20.. Multiple factors, the exact nature of which has not yet been determined, govern the reaction to bunt of wheat.

21. Production of resistant varieties suitable for the prairie provinces of Canada offers a very promising means for reducing the losses due to bunt of wheat.

References

1. AAMODT, O. S. Breeding wheat for resistance to physiologic forms of stem rust. J. Am. Soc. Agron. 19: 206-218. 1927.
2. BRENTZEL, W. E. and RALPH W. SMITH. Varietal resistance of spring wheats to bunt. N. Dak. Sta. Bul. 231. 1929.
3. BRESSMAN, E. N. Physiologic forms of bunt of wheat and varietal resistance. Phytopathology, 21: 108. 1931 (Abstract).
4. BRIGGS, F. N. Inheritance of resistance to bunt, *Tilletia tritici* (Bjerk.) Winter, in wheat. J. Agr. Research, 32: 973-990. 1926.
5. BRIGGS, F. N. Factors which modify the resistance of wheat to bunt, *Tilletia tritici.* Hilgardia 4: 175-184. 1929.
6. BRIGGS, F. N. Inheritance of resistance to bunt, *Tilletia tritici*, in White Odessa wheat. J. Agr. Research, 40: 353-359. 1930.
7. BRIGGS, F. N. Inheritance of the second factor for resistance to bunt, *Tilletia tritici*, in Hussar wheat. J. Agr. Research, 40: 225-232. 1930.
8. BRIGGS, F. N. Inheritance of resistance to bunt, *Tilletia tritici*, in hybrids of White Federation and Banner Berkeley wheats. J. Agr. Research, 42: 307-313. 1931.
9. COONS, G. H. Varietal resistance of winter wheats to *Tilletia levis* (Abstract). Phytopathology, 14: 38-39. 1924.
10. DILLON-WESTON, W. A. R. Resistance of wheat varieties to bunt (*Tilletia caries*). Nature, 123: 243. 1929.
11. FARIS, J. A. Factors influencing the infection of wheat by *Tilletia tritici* and *T. laevis.* Mycologia, 16: 259-282. 1924.
12. FARIS, J. A. Physiological specialization of *Ustilago hordei.* Phytopathology, 14: 537-557. 1924.
13. FARRER, W. Results of the Lambrigg bunt experiments of 1900. Agr. Gaz. N.S. Wales, 12: 419-430. 1901.
14. FARRER, W. Report of the wheat experimentalist. Agr. Gaz. N.S. Wales, 15: 1047-1050. 1904.
15. FARRER, W. Some notes for wheat-growers. Agr. Gaz. N.S. Wales, 16: 462-472. 1905.
16. FLOR, H. H. Indications of heterothallism in *Tilletia triciti.* Phytopathology, 21: 107. 1931. (Abstract).
17. GAINES, E. F. Comparative smut resistance of Washington wheats. J. Am. Soc. Agron. 10: 218-222. 1918.
18. GAINES, E. F. The inheritance of resistance to bunt or stinking smut of wheat. J. Am. Soc. Agron. 12: 124-132. 1920.
19. GAINES, E. F. Genetics of bunt resistance in wheat. J. Agr. Research, 23: 445-480. 1923.
20. GAINES, E. F. New physiologic forms of *Tilletia levis* and *Tilletia tritici.* Phytopathology, 18: 579-588. 1928.
21. GÜSSOW, H. T. and I. L. CONNERS. Smut diseases of cultivated plants. Their cause and control. Dom. of Can. Dept. of Agr. Bull. 81. 1927.
22. HANNA, W. F. and W. POPP. Bunt of wheat in Western Canada. Sci. Agr. 11: 200-207. 1930.
23. HASKELL, R. J. and E. G. BOERNER. How much stinking smut in the field makes grain smutty? Official Record U.S. Dept. Agr. 10: 202. 1931.
24. HEALD, F. D. and E. F. GAINES. The control of bunt or stinking smut of wheat. State Coll. of Wash. Agr. Exptl. Sta. Bull. 241. 1930.
25. HEALD, F. D. and H. M. WOOLMAN. Bunt or stinking smut of wheat. Wash. Agr. Exptl. Sta. Bul. 126. 1915.
26. HOLTON, C. S. A probable explanation of recent epidemics of bunt in durum wheats. Phytopathology, 20: 353-357. 1930.
27. HOLTON, C. S. The relation of physiologic specialization in *Tilletia* to recent epiphytotics of bunt in durum and Marquis wheats. Phytopathology, 21: 687-694. 1931.

28. JOHNSTON, C. O. Wheat bunt investigations in Kansas. Phytopathology, 14:37. 1924. (Abstract).

29. KNIEP, H. Untersuchgen uben den Antherenbraud (*Ustilago violacea* Pers.). Ein Beitrag zum Sexualitatsproblem. Z. Botan. 11: 275-284. 1919.

30. LARMOUR, R. K. The relative quality of Marquis, Garnet and Reward wheats grown in Saskatchewan. Univ. of Sask. Bul. 49. 1931.

31. MCFADDEN, EDGAR S. A successful transfer of Emmer characters to *vulgare* wheat. J. Am. Soc. Agron. 22: 1020-1034. 1930.

32. REED, G. M. Varietal susceptibility of wheat to *Tilletia laevis* Kühn. Phytopathology, 14: 437-450. 1924.

33. REED, G. M. Physiologic races of oat smuts. Am. J. Botany, 11: 483-492. 1924.

34. REED, G. M. Plant pathology. Varietal resistance and physiologic specialization in bunt of wheat. Brooklyn Botan. Garden Record, 16: 37-45. 1927.

35. REED, G. M. Physiologic races of bunt of wheat. Am. J. Botany, 15: 157-170. 1928.

36. REICHERT, I. Comparative bunt resistance of wheat in Palestine. Inst. Agr. and Nat. Hist.; Agr. Exptl. Sta. (Palestine) Bul. 9. 1928.

37. RODENHISER, H. A. and E. C. STAKMAN. Physiologic specialization in *Tilletia levis* and *Tilletia tritici*. Phytopathology, 17: 247-253. 1927.

38. RODENHISER, H. A. Physiologic specialization in some cereal smuts. Phytopathology, 18: 955-1003. 1928.

39. SAMPSON, KATHLEEN. The relative resistance of wheat varieties to bunt (*Tilletia tritici*). Welsh J. Agr. 3: 180-196. 1927.

40. STAKMAN, E. C., E. B. LAMBERT and H. H. FLOR. Varietal resistance of spring wheats to *Tilletia levis*. Minn. Studies in Plant Sci. No. 5. 307-317. 1924.

41. STEPHENS, D. E. and H. M. WOOLMAN. The wheat bunt problem in Oregon. Ore. Agr. Exptl. Sta. Bul. 188. 1922.

42. TISDALE, W. H., J. H. MARTIN, F. N. BRIGGS, W. W. MACKIE, H. M. WOOLMAN, D. E. STEPHENS, E. F. GAINES, F. J. STEVENSON. Relative resistance of wheat to bunt in Pacific Coast States. U.S. Dept. Agr. Bul. 1299. 1925.

43. VAVILOV, N. Immunity of plants to infectious diseases. Annales de l'Acad. Agron. Petrovskoe. 239 pp. 1918. (Russian with English summary).

44. WOOLMAN, H. M. and H. B. HUMPHREY. Summary of literature on bunt, or stinking smut, of wheat. U.S. Dept. Agr. Bul. 1210. 1924.

ACCESSORY FOOD SUBSTANCES FOR OSMOPHILIC YEASTS

I. A BIOACTIVATOR IN HONEY STIMULATING FERMENTATION[1]

BY A. G. LOCHHEAD[2] AND LEONE FARRELL[3]

Abstract

Honey was found to contain an active principle which stimulated fermentation by certain osmophilic yeasts of the genus *Zygosaccharomyces* in synthetic media. The substance is dialyzable, insoluble in ether and acetone, not precipitated by 85% alcohol, resistant to heating in acid solution and non-volatile. The activating effect of honey is impaired by heating in alkaline solution and by prolonged exposure to moderate dry heat. The active principle may be separated into two fractions by selective adsorption by charcoal. The adsorbed fraction, which may be recovered by elution with alcohol, and the unadsorbed fraction are relatively inert alone, the presence of both being necessary for the active stimulation of fermentation.

Introduction

As the result of a number of investigations (2, 4, 6, 7, 8) honey fermentation is now recognized as being due to the action of osmophilic yeasts capable of developing in sugar solutions of concentrations sufficient to suppress the growth of most common types. Such sugar-tolerant yeasts have been isolated, not only from fermented honey, but also from such sources of infection as floral nectar (4), the bodies of bees (9), soil from apiary ground (3) and utensils used for extraction (4).

The belief that normal honey is regularly infected is supported by the results of a study of 191 samples from various parts of Canada (5). In all cases sugar-tolerant yeasts were found, though in widely varying numbers. The tendency to ferment was found to increase with increasing yeast infection, while a study of the chemical composition indicated moisture as the outstanding factor affecting fermentation. While yeast infection and moisture were very important predisposing causes, certain anomalous cases of fermentation with comparatively low, and non-fermentation with relatively high moisture and yeast contents, suggested that other factors were also concerned. In the absence of any further explanation from the chemical analysis, the possible presence in honey of some unidentified factor affecting yeast activity suggested itself. In further support of this hypothesis were observations that little or no fermentation occurred with honey-fermenting yeasts in synthetic dextrose broth, whereas the addition of some "natural" nutrient such as yeast extract, wort, etc., resulted in active fermentation. Since the addition of honey produced a similar effect, a more detailed investigation was made of the factor responsible.

[1] *Manuscript received October 8, 1931.*
Contribution from the laboratories of the Division of Bacteriology, Dominion Experimental Farms, Ottawa, with the co-operation of the National Research Council of Canada.

[2] *Dominion Agricultural Bacteriologist, Department of Agriculture.*

[3] *Chemist, Associate Committee on Honey Research, National Research Council of Canada.*

Effect of Addition of Honey to Synthetic Nutrient Solution

The synthetic nutrient solution used was intended to approximate the composition of honey and was based on the average analysis of 20 American honeys reported by Browne (1). One litre of solution contained: sucrose, 19.0 gm.; dextrin, 15.0 gm.; asparagin, 1.4 gm.; K_2HPO_4, 1.0 gm.; $MgSO_4$, 0.5 gm.; NaCl, 0.1 gm.; $CaCl_2$, 0.1 gm. and malic acid, 1.2 gm. Dextrose was used as the major source of carbohydrate, the amount varying in the preliminary qualitative tests from 30%-60%. This was later standardized to 40% for most of the quantitative experiments. The medium, after sterilization, had a pH value of 4.0-4.2, approximating that of normal honey. For the tests, cultures of honey-fermenting yeasts were used, the inoculum being prepared from agar slope cultures suspended in dextrose broth of similar concentration to that of the nutrient solution employed.

Preliminary Qualitative Tests

Preliminary tests were conducted to note the effects of adding to the basic nutrient solution varying percentages of honey solution of equal sugar concentration. Repeated experiments showed that the addition of honey solution in as small a proportion as 1 cc. to 99 cc. of synthetic solution noticeably shortened the period before the visible onset of fermentation. The outstanding difference in composition between the honey solution and the synthetic nutrient solution is the presence of levulose in the honey, amounting normally to slightly over half of the reducing sugars contained.

To determine whether its content of levulose was responsible for the accelerated fermentation noted with the addition of honey, two sets of experiments were arranged, in one of which honey, and in the other, levulose was added in varying proportions to the basic solution. The amount of levulose added in each case was equal to the levulose contained in the added honey of the corresponding test. Similar tests were made with four honey-fermenting yeasts, cultures J7, E6, D1, M1, and also with a mixture of the four. From the data in Table I, showing the results with culture D1, there is an indication of the presence in honey of some factor hastening the onset of fermentation. This factor does not appear to be levulose.

Quantitative Tests

In the preliminary tests the criterion of activation was the observed time of the onset of gas production. Further experiments, however, were made on a more strictly quantitative basis, for which purpose 300-cc. Erlenmeyer flasks fitted with Alwood fermentation valves were used, each containing 100 cc. of medium. The basic synthetic medium employed was that described above and contained for most tests, 40% dextrose, though a medium containing 60% was used for the first series. Solutions containing different proportions of honey were prepared by adding to the basic solutions varying amounts of a honey solution diluted to contain the same amount of sugar calculated as invert sugar. Thus a medium containing 5% honey solution is one prepared by adding 5 cc. of honey solution to 95 cc. of basic nutrient solution both of equal

TABLE I

EFFECT OF ADDITION OF HONEY AND LEVULOSE UPON FERMENTATION OF SYNTHETIC SOLUTION

Substance added	Onset of fermentation (days), duplicate tubes						
	3	4	5	6	7	8	10
Control	− −	− −	− −	− −	− −	+ +	
1% Honey sol'n.	− −	− −	− −	− −	+ +		
5% Honey sol'n.	− −	+ +					
10% Honey sol'n.	− −	+ +					
20% Honey sol'n.	− −	+ +					
30% Honey sol'n.	− −	+ +					
Levulose of 1% honey sol'n.	− −	− −	− −	− −	− −	− −	+ +
Levulose of 5% honey sol'n.	− −	− −	− −	− −	− −	− −	+ +
Levulose of 10% honey sol'n.	− −	− −	− −	− −	− −	− −	+ +
Levulose of 20% honey sol'n.	− −	− −	− −	− −	− −	+ +	
Levulose of 30% honey sol'n.	− −	− −	− −	− −	− −	− −	+ +

sugar content. The flasks were sterilized by autoclaving and inoculated with a suspension of yeast culture D1, isolated from fermented honey (4) and classified as *Zygosaccharomyces mellis* of Fabian and Quinet (2). The flasks were incubated at 28° C. and weighed at intervals of three to five days, the loss in weight being considered due to CO_2 evolution, after corrections were made for any change in uninoculated controls. The figures given represent averages from duplicate flasks.

The effect of varying percentages of honey solution upon CO_2 production is shown in Tables II and III in which are presented the results from tests with solutions containing respectively 60% and 40% sugar. Fig. 1 and 2 show

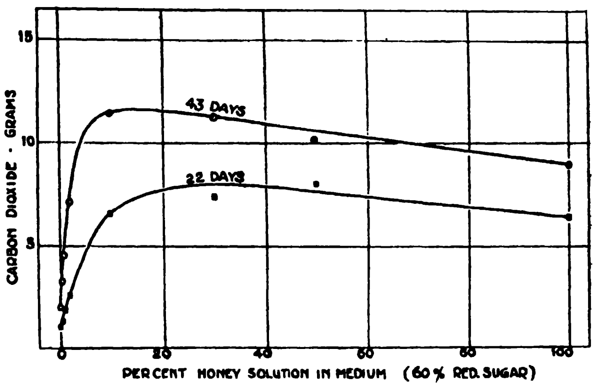


FIG. 1. *Effect of concentration of honey solution on fermentation of synthetic dextrose medium (60% sugar).*

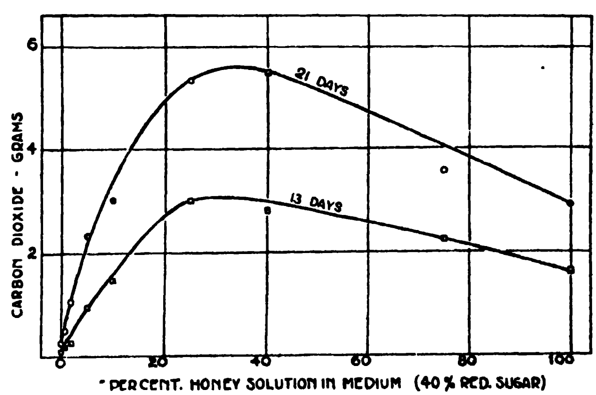


FIG. 2. *Effect of concentration of honey solution on fermentation of synthetic dextrose medium (40% sugar).*

the effect of honey concentration upon total CO_2 production after different periods of time. From these it will be observed that the addition of relatively small proportions of honey has a very marked effect, the curves rising sharply to a maximum and then gradually declining as the nutrient medium approaches a composition of 100% honey solution. To account for this decline two possibilities suggest themselves: the presence of toxic substance in honey or a lack of yeast nutrient materials in honey as compared with the basic solution.

TABLE II

EFFECT OF CONCENTRATION OF HONEY SOLUTION ON FERMENTATION OF SYNTHETIC MEDIUM (60% SUGAR)

% Honey solution	Loss in weight (gm.)										
	5 days	9 days	12 days	19 days	22 days	26 days	29 days	33 days	36 days	40 days	43 days
0	0.25	0.25	0.4	0.65	1.05	1.2	1.1	1.25	1.65	2.0	2.05
0.5	0.05	0.35	0.45	0.9	1.25	1.65	1.75	1.8	2.6	2.95	3.2
1.0	0.2	0.4	0.55	1.35	1.85	2.35	2.55	2.95	3.65	4.25	4.5
2.0	0.2	0.5	0.8	2.0	2.65	3.6	4.2	5.0	5.95	6.75	7.15
10	0.45	1.7	2.35	5.2	6.55	7.95	8.75	9.65	10.5	11.15	11.45
30	0.5	1.75	2.7	5.9	7.3	8.6	9.2	9.8	10.5	10.9	11.2
50	0.35	2.1	3.0	6.9	8.0	8.6	8.95	9.2	9.65	10.1	10.15
100	0.4	2.05	2.8	5.65	6.3	7.25	7.75	7.75	8.45	8.85	8.95

TABLE III

EFFECT OF CONCENTRATION OF HONEY SOLUTION ON FERMENTATION OF SYNTHETIC MEDIUM (40% SUGAR)

% Honey solution	Loss in weight (gm.)						
	3 days	6 days	9 days	11 days	13 days	17 days	21 days
0	0.0	0.05	*0.05*	*0.1*	0.1	0.2	0.25
0.5	0.05	*0.1*	0.1	*0.1*	0.25	0.3	0.55
1	*0.15*	*0.25*	*0.1*	*0.2*	0.15	0.35	0.5
2	0.0	*0.2*	*0.05*	*0.15*	0.25	0.55	1.05
5	*0.15*	*0.2*	0.15	0.4	0.95	1.6	2.35
10	0.0	0.15	0.6	0.9	1.45	2.2	3.0
25	*0.05*	0.35	1.3	1.8	3.0	4.35	5.35
40	0.0	0.25	1.05	1.65	2.8	4.45	5.5
75	0.05	0.3	1.2	1.7	2.25	3.0	3.55
100	*0.15*	0.05	0.06	1.0	1.6	2.25	2.9

NOTE:—*Figures in italics indicate gain in weight.*

This point was investigated in an experiment in which, in addition to the regular series, flasks were prepared with solutions in which extra nutrient salts were added to the dextrose broth-honey mixtures, in such proportions that the total basic nutrient material was the same in each case, and equal to the amount in the basic synthetic solution used as control. Thus when 75 cc. of honey solution was added to 25 dextrose broth, the same amount of extra nutrients was added as in 75 cc. dextrose broth. The results, presented in

Table IV and Fig. 3, lead to the conclusion that the decreasing fermentation noted with the higher proportions of honey in the honey-dextrose broth mixtures is due to a lack of yeast nutrients in the honey which are present in the basic solution chosen. In the case of the solutions with added nutrients (B in Fig. 3) the medium contained, in addition to those added, the nutrients of honey itself, which naturally increased with increasing proportions of honey. This circumstance, however, is not believed to account for the continued rise in curve B up to 100% honey, in view of the results given in Table V and also because the addition of honey ash, as shown later, had no effect in increasing fermentation.

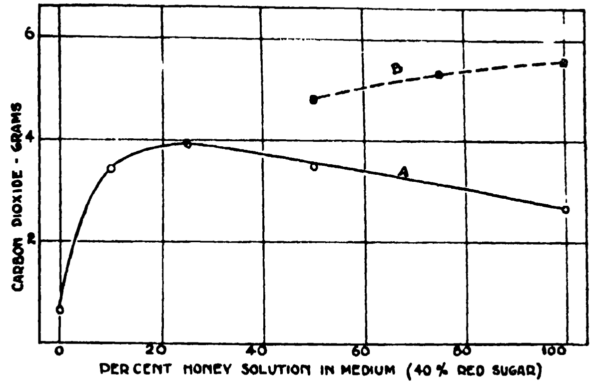


FIG. 3. *Effect of added nutrients on action of honey in stimulating fermentation in synthetic dextrose medium.*

TABLE IV

EFFECT OF ADDED NUTRIENTS ON ACTION OF HONEY

Composition of medium	Loss in weight (gm.)				
	4 days	7 days	10 days	12 days	14 days
Control	0.15	0.05	0.1	0.35	0.65
10% Honey solution	0.4	1.3	2.25	2.85	3.4
25% Honey solution	0.6	1.6	2.65	3.25	3.9
50% Honey solution	0.55	1.35	2.25	2.9	3.45
100% Honey solution	0.45	1.0	1.65	2.05	2.65
50% Honey solution + nutrients	0.75	1.45	3.1	3.95	4.8
75% Honey solution + nutrients	0.9	2.15	3.45	4.4	5.3
100% Honey solution + nutrients	0.7	2.2	3.7	4.75	5.55

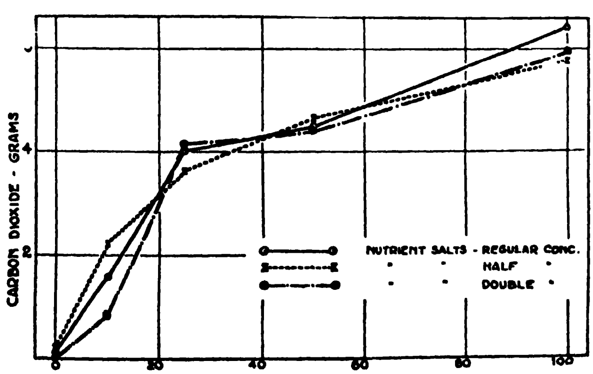


FIG. 4. *Effect of concentration of basic nutrients on action of honey upon fermentation.*

To note the effect of varying the concentration of nutrients chosen for the basic solution, a further experiment was carried out. The influence of various proportions of honey solution added to dextrose broth was noted by preparing different series of solutions in which the concentration of nutrients ranged from one-half to twice that

of the standard medium. Dextrose, sucrose, dextrin and acid remained the same throughout, the mineral salts and asparagin varying as indicated in Table V. The results, summarized in this table and in Fig. 4, indicate that varying the nutrients in the basic medium within the range examined makes little or no difference in the fermentation, and suggest that the basic medium chosen is adequate for the purpose.

TABLE V

EFFECT OF CONCENTRATION OF BASIC NUTRIENTS ON ACTION OF HONEY UPON CONCENTRATION

% Honey	Concentration of basic nutrients	Loss in weight (gm.)				
		5 days	8 days	10 days	12 days	14 days
0% (Control)	×½	0.0	*0.05*	0.05	0.0	0.15
	×1	*0.05*	*0.05*	0.05	0.05	0.1
	×1½	0.0	*0.05*	0.05	0.0	0.1
	×2	*0.05*	0.0	0.05	0.0	0.0
10%	×½	0.05	0.45	0.15	1.75	2.2
	×1	0.05	0.15	0.65	1.10	1.55
	×1½	*0.05*	0.05	0.35	0.65	1.3
	×2	*0.05*	0.05	0.25	0.40	0.8
25%	×½	0.45	1.35	2.2	2.85	3.6
	×1	0.35	1.45	2.45	3.35	4.05
	×1½	0.15	1.10	2.15	3.0	3.7
	×2	0.05	0.9	2.1	3.3	4.15
50%	×½	0.75	2.2	3.25	3.95	4.65
	×1	0.5	2.0	3.05	3.85	4.5
	×1½	0.25	1.7	2.8	3.85	4.35
	×2	0.2	1.3	2.65	3.75	4.45
100%	×½	1.0	2.7	3.95	5.1	5.75
	×1	1.1	3.25	4.8	5.9	6.45
	×1½	0.8	3.15	4.75	5.9	6.4
	×2	0.9	3.1	4.45	5.45	5.95

NOTE:—*Figures in italics indicate gain in weight.*

Characteristics of Bioactivator

With the object of obtaining further knowledge of the substance present in honey stimulating fermentation, additional experiments were made to study the properties of the so-called "bioactivator".

The effect of treatment with adsorbent materials was noted in a preliminary test in which a 90% honey solution was treated with infusorial earth and charcoal* respectively. The honey was boiled with the adsorbent for 10 min., filtered, tubed, sterilized and inoculated with yeasts from fermented honey. It was found that whereas the controls showed fermentation after seven days, the onset of fermentation of honey treated with infusorial earth was delayed but one day, and of that treated with charcoal, five to seven days.

**The source of the charcoal in this case was unknown. Later tests showed that the adsorptive action of different charcoals varied greatly. We noted little or no effect with Merck's animal charcoal, tested on pH ranges from 2.6-7.4, though with Merck's medicinal charcoal, described as blood charcoal, and used for later tests, almost complete inactivation of honey was obtained.*

Effect of Various Treatments

A more extended series of tests was made with a 50% honey solution to note the effect of various methods of handling upon the bioactivator. The activation was observed by adding the treated honey or fractions in 4% proportion to synthetic dextrose solution as previously described, and noting the time of onset of fermentation. All test portions, before addition to the basic solution, were made up to equivalent amounts of original honey. Observations were made of the effect of:

(1) Boiling with medicinal charcoal and fuller's earth.
(2) Dialyzing through parchment membrane.
(3) Drying of pure honey in air at 75° C. for six days.
(4) Autoclaving for one hour at 15 lb. pressure at pH = 4.2 (normal reaction), in neutral solution, and in alkaline solution, final adjustment being made to pH = 4.2.
(5) Distilling.
(6) Treating with lead acetate added to alkaline honey solution, filtering, deleading after filtration and adjusting to pH = 4.2.
(7) Treating with ether, alcohol 95% (to give an 85% solution), and acetone.
(8) Ashing honey.

TABLE VI

EFFECT OF VARIOUS TREATMENTS ON BIOACTIVATOR IN HONEY

Substance added	Onset of fermentation (days)							
	3	4	5	6	7	8	10	13
Control—basic solution	– –	– –	– –	– –	– –	– –	– –	– –
Normal honey	– –	+ +						
Charcoal filtrate	– –	– –	– –	– –	– –	– –	– –	– –
Fuller's earth filtrate	– –	+ +						
Dialysate	– –	+ +						
Air-dried honey	– –	– –	– –	– –	– –	+	+	
Autoclaved honey-acid	– –	– –	+ +					
Autoclaved honey-neutral	– –	– –	– –	+ +				
Autoclaved honey-alkaline	– –	– –	– –	– –	+ +			
Honey distillate	– –	– –	– –	– –	– –	– –	– –	– –
Residue from distillation	– –	– –	+ +					
Lead filtrate	– –	– –	+ +					
Alcohol pp't.	– –	– –	– –	– –	– –	– –	– –	– –
Alcohol filtrate	– –	– –	+ +					
Ether extract	– –	– –	– –	– –	– –	– –	– –	– –
Ether residue	– –	– –	+ +					
Acetone extract	– –	– –	– –	– –	– –	– –	– –	– –
Acetone residue	– –	– –	+ +					
Honey ash	– –	– –	– –	– –	– –	– –	– –	– –

The results, summarized in Table VI, suggest that under the experimental conditions the activating substance in honey is affected by adsorption by charcoal though not by fuller's earth, and is dialyzable. It is insoluble in ether and acetone, though soluble in alcohol (85%). It resists ordinary boiling, is not volatile and withstands autoclaving in acid solution though proving less stable

in alkaline solution. It is noticeably affected, however, by exposure to moderate dry heat. It appears to be absent from honey ash, and is therefore considered to be organic in nature.

Fractionation of Bioactivator

With the apparent adsorption of the activating material by charcoal, attempts were made to recover this by elution with various solvents, for which purpose 1% acetic acid, 1% ammonium hydrate and 95% alcohol were used.

Honey solution of 40% reducing sugar was boiled for 10 min. with 2% medicinal charcoal (Merck), and filtered. The charcoal was removed from the filter and washed with warm water three times. It was then treated with the reagent (100 cc. per 100 cc. of original honey solution) for two hours, after which it was again filtered. In the preliminary trials the filtrate was evaporated to 10 cc. volume, 1 cc. being added to 25 cc. synthetic dextrose solution to note the effect on fermentation. It was observed that although the alcoholic eluate showed a slight activation when added to the basic solution, yet this was much more pronounced when in addition, the solution contained a small amount of the filtrate from the charcoal treatment. The filtrate alone, as previously shown, was relatively inert. It was furthermore noted that the elution with alcohol was much more effective than that with acetic acid or ammonia, and consequently for subsequent tests alcohol was used as standard reagent for elution.

To throw additional light on the apparent existence of two fractions in honey concerned with activation, one of which is adsorbable by charcoal, further quantitative tests were included. Honey was treated with medicinal charcoal as described above and the elution with alcohol similarly conducted. The alcoholic filtrate was evaporated to a few cc. and then made up with distilled water to the same volume as the original honey solution. The single and combined effects of the eluate thus prepared and the charcoal filtrate were compared with that of untreated honey. Additions were made of 10-cc. amounts to the synthetic dextrose solution in Erlenmeyer flasks as previously described, the total volume being 100 cc. The sugar concentration was maintained at 40%.

TABLE VII

EFFECT OF FRACTIONS FROM CHARCOAL TREATMENT

Material added	Loss in weight (gm.)			
	5 days	8 days	11 days	14 days
Control (basic solution)	0.05	0.2	0.35	0.25
Honey (untreated)	0.3	1.45	2.55	3.25
Charcoal filtrate	0.0	0.25	0.35	0.3
Eluate	0.0	0.3	0.3	0.3
Filtrate+eluate	0.2	0.95	1.5	1.85

Results from a typical experiment are outlined in Table VII and Fig. 5, and support the belief that there is in honey a material stimulating fermentation which may be fractionated, giving one portion adsorbable by charcoal and one which is not. Both of these portions appear necessary. From the data it might be inferred, however, that these portions are not different, and that adsorption and elution were very incomplete. In this case filtrate and eluate might contain the same substance, but each in such small quantity as to be ineffective when added alone.

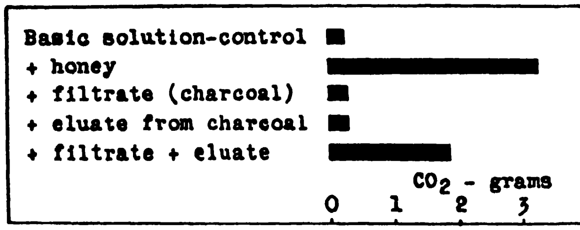


FIG. 5. *Effect of fractions from charcoal treatment on fermentation.*

With this consideration in view, a series was prepared in which, in addition to the usual amounts, double quantities of filtrate and eluate were also used. The results, presented in Table VIII and Fig. 6, support the belief that the stimulation of fermentation by honey depends upon the presence of at least two active principles. The fact that increasing the amount of either filtrate or eluate is without definite effect leads to the view that the stimulation observed when both filtrate and eluate are present is the result of activating bodies of different kinds rather than a simple additive effect.

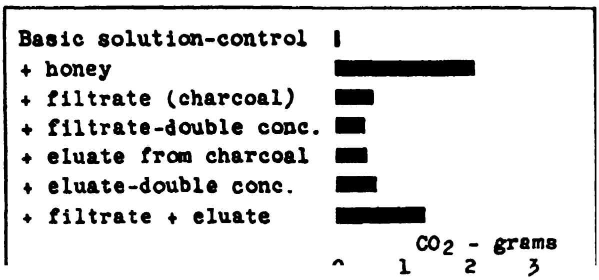


FIG. 6. *Effect of concentration of fractions from charcoal treatment on fermentation.*

TABLE VIII

EFFECT OF CONCENTRATION OF FRACTIONS

Material added	Loss in weight (gm.)			
	4 days	8 days	11 days	14 days
Control (basic solution)	0.0	0.0	*0.1*	0.0
Honey (untreated)	0.25	0.95	1.6	2.1
Charcoal filtrate	0.0	0.15	0.3	0.55
Charcoal filtrate (double)	0.1	0.2	0.3	0.4
Eluate from charcoal	0.1	0.1	0.3	0.45
Eluate (double)	0.1	0.2	0.35	0.6
Filtrate+eluate	0.15	0.65	1.05	1.3

NOTE:—*Figures in italics indicate gain in weight.*

References

1. Browne, C. A. U.S. Dept. of Agr., Bur. of Chem., Bull. 110. 1908.
2. Fabian, F. W. and Quinet, R. I. Mich. Agr. Exp. Sta. Tech. Bull. 92. 1928.
3. Lochhead, A. G. and Farrell, Leone. Can. J. Research, 3: 51-64. 1930.
4. Lochhead, A. G. and Heron, D. A. Dom. of Can. Dept. Agr. Bull. 116, n.s. 1929.
5. Lochhead, A. G. and McMaster, N. B. Sci. Agric. 11: 351-360. 1931.
6. Marvin, G. E. J. Econ. Entomol. 21: 363-370. 1928.
7. Nussbaumer, T. Z. Nahr. Genussm. 20: 272-277. 1910.
8. Richter, A. A. Mycol. Centr. 1: 67-76. 1912.
9. Wilson, H. F. and Marvin, G. E. J. Econ. Entomol. 22: 513-517. 1929.

ACCESSORY FOOD SUBSTANCES FOR OSMOPHILIC YEASTS

II. COMPARISON OF HONEY BIOACTIVATOR WITH BIOS[1]

By Leone Farrell[2] and A. G. Lochhead[3]

Abstract

A bioactivator from honey, stimulating osmophilic yeasts, was compared with Bios. Complementary fractions from treatment with charcoal were found to exert effects similar to Bios I (inosite) and Bios II of Miller and associates, when tested with the Toronto strain of *Saccharomyces cerevisiae*. Charcoal treatment of honey removes by adsorption Bios II leaving a residue, relatively inert by itself, containing inosite. Inosite, however, does not appear as the active substance in the charcoal filtrate for the strain of osmophilic *Zygosaccharomyces* tested, the growth of this organism being dependent upon the presence of another substance which, though not essential for the Toronto yeast, appears to be present in crude Bios II.

Introduction

In a previous communication (6) data were presented indicating the presence in honey of an active principle capable of stimulating fermentation by certain osmophilic yeasts of the genus *Zygosaccharomyces* in synthetic media. The recognition of this bioactivator naturally suggested a possible relationship with accessory factors found by other workers to be necessary for the normal growth and metabolism of certain strains of *Saccharomyces cerevisiae*. Such factors are generally considered under the term "Bios".

Although but thirty years have elapsed since the introduction of the term Bios by Wildiers (12) to denote the substance regarded by him as indispensable to normal yeast development, there has already appeared a voluminous and polemical literature on the subject. A detailed résumé of the work on Bios up to 1925 has been given by Tanner (10), while more recent contributions to the subject have been summarized by the same writer (11) and by Buchanan and Fulmer (1). In addition, a critical survey of the whole Bios question, together with the most pertinent references, has recently been presented by Miller (8). Many of the divergent results of different workers have apparently been due to such causes as the use of ingredients of varying degrees of purity, the employment of different strains of yeast, neglect to prevent contamination by other micro-organisms, etc. Now, however, it is generally agreed that for certain strains of yeast at least, Bios is necessary for development. Other strains, apparently, are able to grow without such accessory substances.

The properties of the substance in honey capable of stimulating the fermentative action of the osmophilic yeasts tested corresponded very closely with those listed by Wildiers. The bioactivator of honey, like Wildiers' Bios, is dialyzable, soluble in water and 80-85% alcohol, but insoluble in ether.

[1] *Manuscript received October 8, 1931.*
Contribution from the laboratories of the Division of Bacteriology, Dominion Experimental Farms, Ottawa, with the co-operation of the National Research Council of Canada.

[2] *Chemist, Associate Committee on Honey Research, National Research Council of Canada.*

[3] *Dominion Agricultural Bacteriologist, Department of Agriculture.*

It is resistant to heating in acid solution, although injured in alkaline solution, Since it is not found in honey ash, it is, like Bios, organic in nature. Additional tests showed the honey bioactivator to be non-volatile. Although it is capable of withstanding autoclaving in acid solution (pH = 4.2 approx.), yet prolonged exposure to moderate dry heat (75° C.) noticeably affected it.

From such materials as autolysed yeast and rice polishings a number of workers have isolated definite chemical compounds having an effect upon yeast growth comparable with that of Wildiers' Bios. Thus Eddy, Kerr and Williams (4) described the isolation from yeast of a crystalline compound, $C_5H_{11}NO_3$, of definite melting point, while from rice polishings the isolation of a compound of formula $C_{10}H_8N_4$ with Bios effect has been reported by Kinugasa (5). That the active substance in honey is not similar to these, however, is indicated by the fact that, as previously shown (6), it may be separated into two complementary fractions by treatment with charcoal. The fractions are relatively inert when used alone, though together they noticeably stimulate the fermentation caused by strains of *Zygosaccharomyces*.

Previous workers have reported the fractionation of substances showing Bios activity. From wort and malt combings Lucas (7) succeeded in separating two fractions, called Bios I and Bios II. The former was precipitated while Bios II remained in solution upon treatment with barium hydroxide in alcoholic solution. Neither Bios I nor Bios II alone had any great influence on the growth of the strain of *Saccharomyces cerevisiae* used, though together they greatly increased the yeast crop when added to the basic salts-sugar solution. Bios I has since been identified as inactive inosite by Eastcott (3). The fractionation of yeast extract into two portions by treatment with fuller's earth has been reported by Williams, Wilson and Von der Ahe (15). The fractions obtained showed individually very little Bios activity though a pronounced effect resulted with the two together when used with their strain of yeast. Further work by Williams, Warner and Roehm (14) on fractions derived from yeast extract supported the view that specific strains of yeast vary in their Bios requirements. This point is further emphasized by the work of Copping (2) whose studies indicated that the need for Bios is dependent upon the type of yeast.

In our studies (6) the stimulating effect of honey on fermentation by the strain of *Zygosaccharomyces* used was almost entirely removed by treatment with charcoal. Narayanan (9), however, noted no adsorptive effect with charcoal (norite), using yeast extract as a source of growth stimulant for his strain of *Saccharomyces cerevisiae*. Similarly Williams and Bradway (13), in testing the effect of their fractions on different yeast strains, report that for Wildiers' original yeast culture the essential growth factor present in yeast extract is not readily adsorbed by fuller's earth, and in their data find no evidence of the multiple nature of Wildiers' Bios. In this connection, however, it may be emphasized that fractionation of a yeast stimulant into complementary portions may greatly depend upon the adsorbent used. With our bioactivator from honey we noted little or no adsorptive effect with fuller's earth, kaolin or Merck's animal charcoal, the last-named being employed at

pH values ranging from 2.6 to 7.4. In such cases the filtrate from the treatment was as active in stimulating fermentation as the untreated honey. Using Merck's "medicinal" charcoal, described as blood charcoal, almost complete inactivation of the honey was effected after filtration, and subsequent tests of the unadsorbed and adsorbed fractions permitted the establishment of the multiple nature of the bioactivator.

Comparison of Honey Bioactivator with Lash Miller's Bios

Through the kindness of Prof. W. Lash Miller a series of tests was made possible with the object of comparing the stimulating effect of our bioactivator with that of Bios I and Bios II of the Toronto workers. One set of experiments was conducted in the Toronto laboratory in which were used the standard methods and the strain of yeast employed by the workers there (7). Materials supplied by us consisted of untreated honey solution, filtrate from the charcoal treatment and adsorbed fraction (eluate) recovered by elution of the charcoal with alcohol (6). The results of this experiment as supplied by Dr. Miller are given in Table I and Fig. 1.

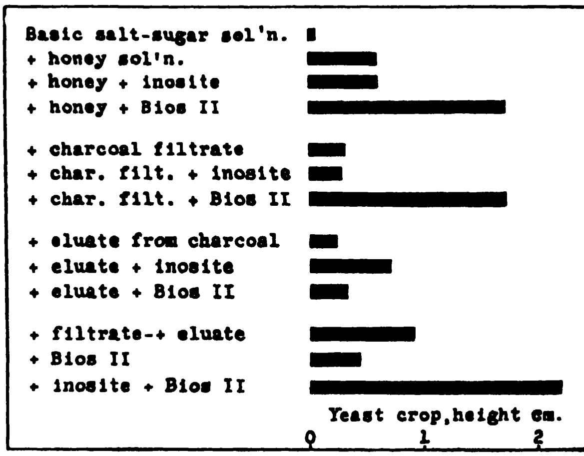


FIG. 1. *Effect of honey bioactivator and Bios upon Saccharomyces cerevisiae (Toronto strain).*

TABLE I

EFFECT OF HONEY BIOACTIVATOR AND BIOS UPON *Saccharomyces cerevisiae* (TORONTO STRAIN)

Substance added	Yeast crop (cm.)	Substance added	Yeast crop (cm.)
Salts-sugar solution (control)	0.05	Eluate from charcoal	0.22
Honey solution	0.57	Eluate+inosite	0.67
Honey+inosite	0.58	Eluate+Bios II	0.32
Honey+Bios II	1.70	Filtrate+eluate	0.88
Charcoal filtrate	0.30	Bios II	0.43
Charcoal filtrate+inosite	0.27	Inosite+Bios II	2.20
Charcoal filtrate+Bios II	1.70		

It will be observed that the bioactivator from honey exerts a Bios effect and that the fractions from the charcoal treatment have a complementary action, characteristic of Bios I and Bios II. The yeast crop from the charcoal filtrate is not increased by adding inosite (Bios I) though noticeably greater

with the addition of Bios II, suggesting that our filtrate contains inosite. With the charcoal eluate, on the other hand, the crop is increased by adding inosite, but not significantly increased with the addition of Bios II. This points to the probable identity of the active substance in our eluate with Bios II, a hypothesis strongly supported by the fact that Bios II is adsorbed by charcoal. It would appear, therefore, that for the Toronto yeast, our filtrate and eluate are qualitatively interchangeable with Bios I and Bios II respectively. The Bios II solution used was apparently much more potent than our charcoal eluate. It may be pointed out, however, that our fractions were purposely diluted to volumes corresponding with the original honey solution.

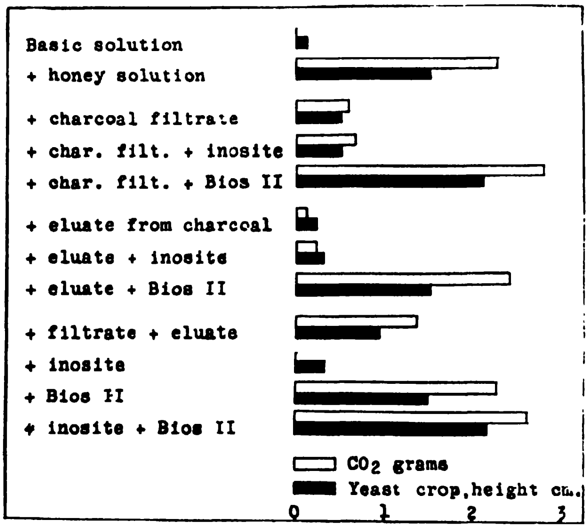


FIG. 2. *Effect of honey bioactivator and Bios upon Zygosaccharomyces mellis.*

Corresponding experiments were conducted in this laboratory with the strain of *Zygosaccharomyces mellis* (Culture D1) isolated from fermented honey, and following the procedure described for earlier tests (6). All solutions contained 40% dextrose, with a 14-day incubation period, previously adopted for osmophilic yeasts. In addition to the CO_2 determinations, estimations of the yeast crop at the end of 14 days were made by measuring the height of the yeast column after centrifuging into a narrow tube 2 cc. of the test solution mixed with 2 cc. of 10% chloracetic acid (7). The inosite and Bios II solution ("crude Bios II") were supplied by Dr. Miller. The results of two separate sets of experiments, each made in duplicate, are shown in Table II and Fig. 2.

From the data it will be seen that the stimulation of the preparations tested was different, with our osmophilic yeast, from the effect produced with the Toronto strain. With our yeast, the yeast crop and CO_2 production with the charcoal filtrate were not significantly increased with inosite, though noticeably so with Bios II. Differing from its effect on the Toronto yeast, however, Bios II, which contains no inosite, has a pronounced effect alone on our yeast. There is thus reason to believe that the growth and fermentative action of our *Zygosaccharomyces* is not dependent on the presence of inosite, which, as the data in Table I indicate, may be contained in the filtrate. This is further supported by its behavior in the presence of inosite and the charcoal eluate. With our yeast no complementary effect was noted, the addition of inosite to eluate having no significant effect over either alone, whereas with the Toronto

TABLE II

EFFECT OF HONEY BIOACTIVATOR AND BIOS UPON *Zygosaccharomyces mellis* (CULTURE D1)

Substance added	Experiment 1		Experiment 2	
	CO_2, gm.	Yeast crop, cm.	CO_2, gm.	Yeast crop, cm.
Basic solution (control)	0.05	0.2	*0.05*	0.05
Honey solution	2.6	1.5	1.9	1.5
Charcoal filtrate	0.55	0.6	0.65	0.4
Charcoal filtrate+inosite	0.7	0.6	0.65	0.4
Charcoal filtrate+Bios II	2.8	2.1	2.8	2.1
Eluate from charcoal	0.2	0.4	0.0	0.05
Eluate+inosite	0.3		0.15	0.3
Eluate+Bios II	2.35	1.45	2.45	1.6
Filtrate+eluate	1.2	0.9	1.5	0.95
Inosite	0.05	0.3	*0.05*	0.35
Bios II	1.75	1.4	2.7	1.6
Inosite+Bios II	2.9	2.55	2.35	1.8

NOTE:— *Figures in italics indicate a gain in weight of flasks.*

yeast, the addition of inosite to eluate resulted in a significant increase in the crop. With our yeast, therefore, inosite is unable to replace the charcoal filtrate as complement to the eluate, further supporting the belief that the active substance contained in our filtrate is not identical with inosite. As inosite and our eluate permit of good growth with the Toronto yeast, this unknown substance is not essential for growth of that strain, though apparently it is present in crude Bios II.

Acknowledgment

The writers wish to express their thanks to Dr. W. Lash Miller of the University of Toronto for having tested their preparations with his strain of yeast and for having kindly furnished them with a supply of Bios I and II.

References

1. BUCHANAN, R. E. and FULMER, E. I. The physiology and biochemistry of bacteria. Vol. 2. Williams and Wilkins. Baltimore. 1930.
2. COPPING, A. M. Biochem. J. 23: 1051-1063. 1929.
3. EASTCOTT, EDNA V. J. Phys. Chem. 32: 1094-1111. 1928.
4. EDDY, W. H., KERR, R. W. and WILLIAMS, R. R. J. Am. Chem. Soc. 46: 2846-2855. 1924.
5. KINUGASA, Y. J. Pharm. Soc. Japan, 48: 539-563. 1928. (Ref. Chem. Ab. 22: 3915. 1928).
6. LOCHHEAD, A. G. and FARRELL, LEONE. Can. J. Research, 5:529-538. 1931.
7. LUCAS, G. H. W. J. Phys. Chem. 28: 1180-1200. 1924.
8. MILLER, W. LASH. J. Chem. Education, 7: 257-267. 1930.
9. NARAYANAN, B. T. Biochem. J. 24: 6-18. 1930.
10. TANNER, F. W. Chem. Rev. 1: 397-472. 1925.
11. TANNER, F. W. The newer knowledge of bacteriology and immunology (Jordan and Falk). Chicago. Chap. 37. 1928.
12. WILDIERS, E. La Cellule, 18: 313-331. 1901.
13. WILLIAMS, R. J. and BRADWAY, E. M. J. Am. Chem. Soc. 53: 783-789. 1931.
14. WILLIAMS, R. J., WARNER, M. E. and ROEHM, R. R. J. Am. Chem. Soc. 51: 2764-2774. 1929.
15. WILLIAMS, R. J., WILSON, J. L. and VON DER AHE, F. H. J. Am. Chem. Soc. 49: 227-235. 1927.

THE USE OF THE PROJECTION MICROSCOPE AND PHOTO-ELECTRIC CELL

II. BLOOD STUDIES[1]

By Alfred Savage[2] and J. M. Isa[3]

Abstract

This is a continuation of observations published in this Journal (4). It deals with an electrical photometer of high sensitivity but unaffected by red light. This is used in conjunction with the projection microscope and applied to a study of red blood cells stained with basic fuchsine. The measurements reported are a complex of staining intensity and area. These are compared with area estimations alone and it is shown that the former constitutes a greater variable than the latter. This is particularly noticeable when dealing with anemic blood. The relationship between primary and secondary anemia of man is touched upon.

Introduction

In 1930 Savage and Jamieson (4) published a preliminary note which indicated that by the combined use of the above instruments it was possible to determine the comparative areas of certain microscopic images more quickly and more easily than by other methods. Their observations were confined to direct measurements of the photo-electric current and, in consequence, the degree of magnification which they employed was limited. With the light at their disposal 500 diameters could not be exceeded. This sufficed for the larger microscopic objects but was not enough for work with the elements of animal tissues. Therefore it is evident that this method must undergo extension before it becomes applicable to problems in physiology and pathology.

Scope

The present account begins where the previous note ended and deals with observations made in the same general manner. The essential difference is that, in this instance, the photo-electric current was amplified by means of a thermionic valve and measured indirectly. Increased magnification of the images was possible because the inevitable decrease in light intensity was offset by the amplification employed.

Apparatus

The relative positions of microscope, illuminating device and photo-electric cell have been described (4). Added electrical equipment consisted of a special thermionic valve and the necessary accessories for operating it.

Fig. 1 indicates the circuit. The valve was a "Pliotron FP-54" designed by Metcalf and Thompson (1). It was operated at the voltages recommended and the plate current was balanced out as by Razek and Mulder (3) and others.

[1] *Manuscript received August 8, 1931.*
Contribution from the Manitoba Agricultural College, Winnipeg, Canada, with financial assistance from the National Research Council of Canada.

[2] *Professor of Animal Pathology, Manitoba Agricultural College.*

[3] *Special Assistant, Department of Bacteriology and Animal Pathology, Manitoba Agricultural College.*

The galvanometer was of the d'Arsonval type. It had a sensitivity of 0.003 microamperes and a period of three seconds. An Ayrton shunt was connected with it.

The grid leak consisted of about five feet of fine glass rod bent to a spiral 6 in. long, dipped in India ink and sealed into a glass tube 2 in. in diameter.

An extensive shield of sheet copper enclosed the entire amplifying apparatus.

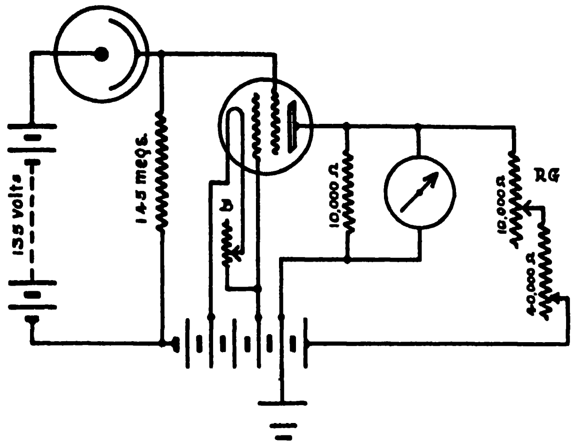


FIG. 1. *Diagram of electrical circuit.*

Operation

When the apparatus was to be used, the current was switched on to both the filament and plant an hour beforehand. Then the photo-electric circuit was closed and the galvanometer balanced at progressive stages until the full current reached it. Finally the lid of the copper shield was closed. Subsequent adjustments to the circuit were made from outside and amounted to very little, the galvanometer "drift" being about 5 cm. per hr.

The lamp for the projection microscope also had to be turned on at least one hour before use. If this were not done the change of its position, due to expansion by heat, appreciably altered the focus and consequently the degree of illumination. It is repeated that this lamp was a spiral filament and therefore not a homogeneous source of light. So far as possible this was overcome by focussing sharply on an incandescent area such as the one indicated in Plate 1, Fig. 1, and by keeping that spot projected into the diaphragm of the photo-cell.

Small changes in the supply current for this lamp (110–115 volts a.c.) were very annoying and could be avoided only by working between the hours of 1 and 5 a.m., when they became negligible.

Behavior of Apparatus

Calibrated with a carbon filament lamp, the photometer had a sensitivity of about 1500 cm. per lumen. The light-current relationship was rectilinear within the range used.

With eosin and fuchsine, it was experimentally verified that dyestuffs in solution transmit light in inverse proportion to the logarithms of their concentration. This is stated because the result of measuring stained objects by this method is a complex product of staining intensity and area. No attempt was made to separate these variables.

Observations on Blood Films

(1) Preparation and Staining

To be satisfactory for this kind of study, blood films had to be made so that nearly every corpuscle was separated from its neighbors by a distance of several times the diameter of each. This was a somewhat exacting prerequisite in the selection of material.

The films were fixed by immersion in a saturated aqueous solution of mercuric chloride for 20 min. and then thoroughly washed in distilled water. They were stained with acid fuchsine (saturated aqueous solution) for 10 min. and washed as above until no trace of stain could be detected in the plasma between the corpuscles. Rapid drying followed, first by waving the slides to remove excess water, then by placing them in an oven at 54° C.

Projection was at 1400 diameters magnification. The diaphragm of the photo-cell was reduced to a $\frac{3}{4}$-in. aperture as a matter of convenience. From this point, the same procedure was followed as in dealing with rust spores (4), observations being made on 500 erythrocytes from each slide.

(2) Normal Blood

A film of normal blood gave galvanometer deflections which, on arrangement into classes, occurred with the frequencies given by Table I. The result is a normal curve having 15 classes.

TABLE I

SIZES AND FREQUENCY DISTRIBUTION OF NORMAL RED BLOOD CELLS AT 1400 DIAMETERS AS INDICATED BY THE PHOTO-ELECTRIC CELL

Deflection of galvanometer in mm.	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Frequency	3	2	6	22	45	78	75	104	70	44	25	12	7	6	1

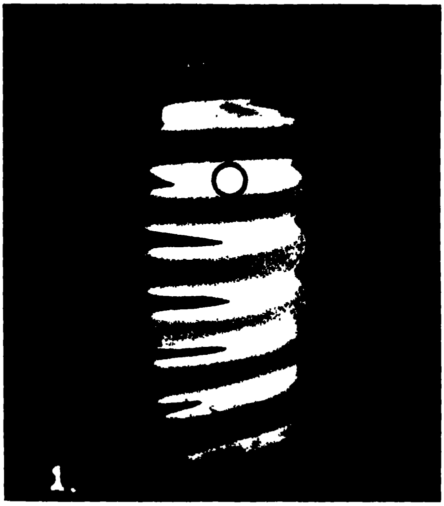
The diameters of 500 corpuscles were then taken at 3000 diameters (by actual measurement of the projected images) and recorded to the nearest 0.5 mm. Similarly arranged, these figures are given in Table II. In this case also, the result is a normal curve, the functions of which are close to those given by Price-Jones (2, p. 10) for measurements made in the same way.

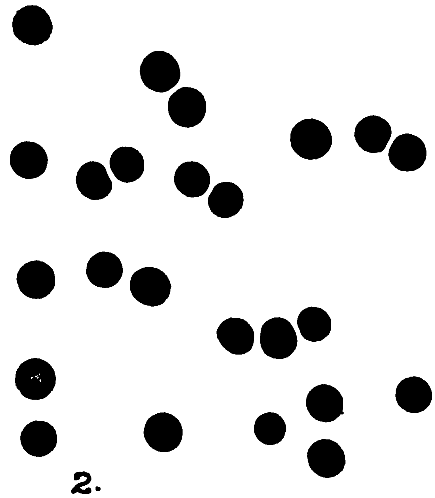
TABLE II

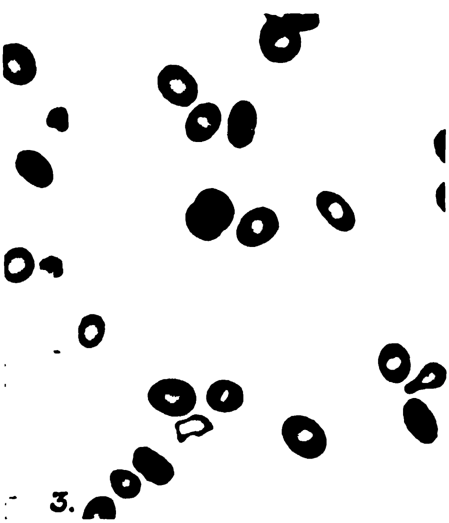
DIAMETERS AND FREQUENCY DISTRIBUTION OF NORMAL RED BLOOD CELLS AS AT 3000 × MAGNIFICATION

Diameter in mm.	17		18		19		20		21		22		23		24		25		26		27
Frequency		1	1	0	2	3	12	13	27	36	49	67	79	63	57	40	31	11	4	2	2

Because of the different number of classes in the two tables given above, they are not strictly comparable in their present form. Even so, one very interesting conclusion may be drawn by comparing them.







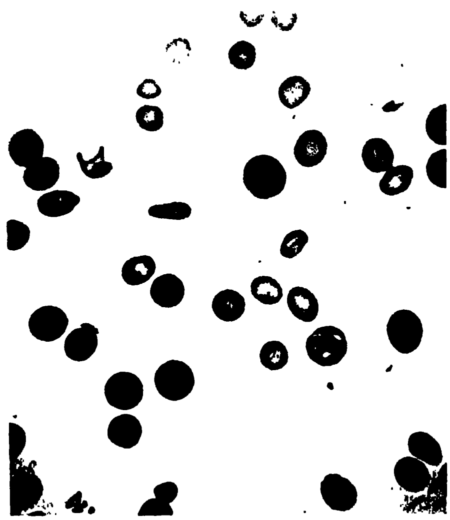


FIG. 1. *Enlarged image of the incandescent filament used as light source. An area similar to that indicated by the small circle was maintained in critical focus.*

FIG. 2. *Normal red blood cells* $\times$ *690.*

FIG. 3. *Red blood cells in primary anemia* $\times$ *690.*

FIG. 4. *Red blood cells in secondary anemia* $\times$ *690.*

Table II deals with diameters of from 17.5 to 27 mm., or varying over a range proportional, roughly, as 1.0 : 1.5. Now the areas of circles vary directly as the squares of their diameters and for this reason one would expect Table I to show an extreme class variation proportional to the squares of these figures, namely as 1 : 2.25. Actually the range is from 6 to 20, or as 1 : 3.3. This is approximately 50% greater than if areas alone were registered by the photo-cell and can be explained only by variations in staining intensity.

(3) Secondary Anemia

By the photo-electric method, a blood film from a case of secondary anemia (3,700,000 red cells per cu. mm.: hemoglobin, 22%) provided the data in Table III.

TABLE III

SIZES AND FREQUENCY DISTRIBUTION OF 500 RED BLOOD CELLS FROM A CASE OF SECONDARY ANEMIA AS INDICATED BY THE PHOTO-ELECTRIC CELL

Deflection of galvanometer in mm.	1	2	3	4	5	6	7	8	9	10	11	12
Frequency	16	57	97	111	102	54	34	15	6	4	3	1

To complete the basis of comparison used in the instance of normal blood, diameter measurements of the corpuscles were made in the same way. In this instance a difficulty was presented by about 50% of the cells being other than circular. They were not grossly distorted but compelled a selection for measurement of only the round ones. The result (to the nearest mm.) is given as Table IV. In addition, the writers satisfied themselves that, so far as the eye could discern, *there were present neither larger nor smaller cells* than those represented by the extreme figures in this table.

TABLE IV

DIAMETERS AND FREQUENCY DISTRIBUTION OF 500 RED BLOOD CELLS FROM A CASE OF SECONDARY ANEMIA, AS AT 3000 X MAGNIFICATION

Diameter in mm.	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Frequency	3	6	10	25	64	89	93	84	69	33	15	3	2	3	1

A comparison of Tables III and IV shows that the proportional range of the latter is as 1 : 2 (or slightly less): of the former, as 1 : *12!* This surprising difference will be discussed later.

(4) Primary (Pernicious) Anemia

The blood film selected as representing this condition had been mounted under a coverslip and was somewhat faded. It was therefore unmounted by immersion in xylol, decolorized with acid alcohol and restained in the same manner as were the other slides studied.

Galvanometer readings obtained from this preparation are given as Table V.

TABLE V

SIZES AND FREQUENCY DISTRIBUTION OF 500 RED BLOOD CELLS FROM A CASE OF PERNICIOUS ANEMIA AS INDICATED BY THE PHOTO-ELECTRIC CELL

Deflection of galvanometer in mm.	1	2	3	4	5	6	7	8	9	10	11	12	13
Frequency	16	29	34	57	83	106	98	44	23	3	3	2	2

Owing to the severe and varied distortions of outline presented by the red cells on this slide, it was considered hopeless to attempt estimation of their areas by means of linear measurements.

Discussion

For comparison the three curves obtained photo-electrically are plotted on the same base line and shown as Fig. 2.

These are all apparently normal frequency distribution curves except that B and C end abruptly over the lowest galvanometer deflection which was recorded. This is probably because no attempt was made to read the apparatus to less than 1.0 mm. Even so, two facts seem to have been brought to light. The first is that the complex measured, namely area × staining intensity, of red blood cells varies more than the areas alone. In the case of normal blood this may be presupposed to have certain limits which have not been determined as yet. Possibly the different ages of the corpuscles affords a partial explanation. However, when pathological blood is considered, other factors enter.

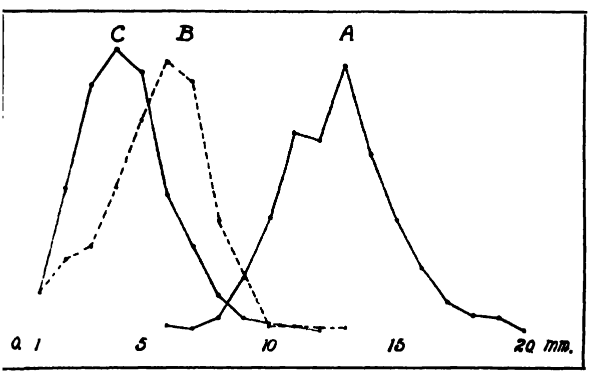


FIG. 2. *Frequency distributions of area × staining intensity of red blood cells in A, normal blood; B, primary anemia and C, secondary anemia as shown by the electrical photometer. 500 cells are represented in each case.*

In ordinary anemia of mild degree, there is a lowering of the hemoglobin content per cell without any noticeable alteration in the size and shape of these elements. This is accompanied by a decreased affinity for the "acid" stains. According to the technique used in these observations the effect of this would be to move the whole curve to the left thus increasing the degree of variation by lowering the value of the mean or, as the writers have chosen to express it, by increasing the proportional spread between the cells of largest and smallest diameter. When the anemia is more marked two other factors

augment this effect. These are: (a) marked variations in the sizes of the cells and, (b) obvious inequalities in their staining reactions. It is suggested that the number of resulting combinations which are possible affords ample explanation for the degree of variation observed.

The second fact concerns a difference between primary and secondary anemia. Evidence is too limited to permit a general conclusion on this point. But, if the slides studied may be considered as typical, it is clear that the above method shows only a difference of degree (which is probably a matter of chance) and not one of kind.

This is in apparent contrast with the findings of Price-Jones (2, p. 10) who studied and measured the red cell diameters in normal blood and in anemias of both types. His figures were arranged to show the frequency distribution of the sizes of these cells in all three conditions. The general conclusion reached by this worker was that the mean red cell diameter is decreased in secondary but *increased in primary anemia.* If to this might be added the fact that in the latter condition the hemoglobin content per cell is usually normal or greater than normal, there would be reason to suppose the above contrast more real than apparent. It is emphasized however that the results are not strictly comparable.

Finally, whether or not the above technique proves of value to the research worker engaged in the study of blood, the writers are of the opinion that no other combination of instruments is capable of giving the same results for analytical and statistical purposes.

Acknowledgments

To Dr. P. A. Macdonald of the Physics Department, University of Manitoba, the writers are indebted for much sound advice regarding the electrical aspects of the problem and for the loan of the Pliotron valve. Dr. S. Meltzer and Dr. D. Nicholson of the Winnipeg General Hospital very kindly supplied the slides of pathological blood. Mr. D. Binnington of the Chemistry Department, Agricultural College, constructed the grid leak.

References

1. Metcalf, G. F. and Thompson, B. J. Phys. Rev. 36: 1489-1494. 1930.
2. Price-Jones, C. Guy's Hospital Reports, 74. 1924.
3. Razek, J. and Mulder, P. J. J. Optical Soc. Am. 18: 460-465. 1928.
4. Savage, A. and Jamieson, M. C. Can. J. Research, 3: 322-326. 1930.

SELECTIVE HEATING BY SHORT RADIO WAVES AND ITS APPLICATION TO ELECTROTHERAPY[1]

By J. C. McLennan[2], F.R.S. and A. C. Burton[3], M.A.

Abstract

In the first part of the paper the theoretical basis of the formulas, given in a previous communication, for the generation of heat in a poorly conducting dielectric which is placed in the field of a high-frequency oscillator, is examined in some detail. Its application to the complicated case met with in medical "radiothermy" is made and it is shown that the analysis applies with satisfactory accuracy to this case, though not to that of electrode diathermy. Prediction of the "selective" effect is possible from a knowledge of the characteristic electrical constants of the body-substances at high frequencies, and by proper choice of wave-length the heating of a particular part might be favored over that of neighboring parts.

In the second part of the paper, experimental work is described which carries verification of the formulas to shorter wave-lengths. Experiments on the heating of meat demonstrate the selective effect and its dependence upon wave-length. The heating of such substances as liver, heart, and the different parts of an egg, are examined experimentally as examples of the various determining factors that are involved.

The theoretical explanation of the effects is considered satisfactory and further developments depend upon the results of *in viva* experiments with shorter wave-lengths than those at present in general use.

Important developments in the application of high frequency electric fields to medicine have been made in the last two or three years. The discovery that a body, placed in the "condenser-field" of a high frequency oscillator of wave-length 50 metres and less, was heated by the oscillations, has been applied extensively in the production of artificial fevers in man. The use of ultra-high frequency in electrotherapy promises to be of very great importance.

The results of a research into the physical causes of the heating and its dependence upon the physical character of the body or bodies being heated, as well as upon the frequency and intensity of the field, have been published (6). The amount of heating was shown to be given, to a close approximation, by the formula,

$$\frac{dT}{dt} - \frac{E^2}{\rho s} f(\theta, \epsilon, K) \frac{x}{1+\left(\frac{2x}{K\nu}\right)^2}, \tag{1}$$

where s = specific heat, ρ = density, x = conductivity in absolute units, K = dielectric constant, and $f(\theta, \epsilon, K)$ is a function of the orientation (θ), the shape (ϵ) and dielectric constant K of the body, whilst E and ν are the intensity and frequency of the high frequency electric field. A paper dealing with the same problem but adopting a rather different method was published at the same time by Pätzold (9), who reached the same general conclusions.

There are two characteristics, peculiar to this method of production of heat, that are significant in its application to medicine. The first is that the heating takes place throughout the interior of the body, the "skin-effect" being

[1] *Manuscript received August 25, 1931.*
Contribution from the Physical Laboratory of the University of Toronto, Toronto, Canada.

[2] *Professor of Physics and Director of the Physical Laboratory, University of Toronto.*

[3] *Holder of a studentship under the National Research Council of Canada.*

of negligible importance in this problem. This makes the use of short wave heating, or "radiothermy", a far more effective method of heat treatment than any external application of heat could be. By its means, the temperature of the blood may rapidly be raised and artificial fevers induced, which before could be produced only by the introduction of irritants such as malarial germs.

The second peculiarity is that the heating is not produced uniformly throughout a heterogeneous body; that there is a selective action, predicted by the formula (1) and shown experimentally to exist. In the special case of a body in which the dielectric constant is the same throughout but the conductivity varies, the maximum heat per unit volume per second is produced in a region for which the relation (2) is true.

$$x = \frac{K\nu}{2}. \tag{2}$$

It may be pointed out that, even if the heat were uniformly produced throughout the body, the difference in the specific heats of different parts would make the resultant rise in temperature a maximum in certain places. The distribution of temperature after heating must then be very different from that produced by the flow of heat into the body from an external source, in which case the temperature gradient must always be of the same sign.

Equation (2) shows that in the choice of the frequency, ν, of the oscillator, we have a means of altering at will the distribution of the heating and consequent temperatures throughout the body. In general, the longer waves, of lower frequency, choose the less conducting portions; the higher frequencies select the portions of greater conductivity.

The application of this selective effect, rather than the general heating effect, has not yet been specifically made to medicine, but the possible usefulness is so great that further examination of the theoretical basis, and further experimental verification has been made, and is described in what follows.

Theory

Since Pätzold (9), Pierce (10), and others have dealt with the problem as a "circuit" problem, in the particular cases represented by the experimental conditions respectively adopted, a general, but quite simple, treatment of the problem by this method is now given. The procedure will be from the simple particular cases to the complexity of the actual conditions of practice.

(a) Condenser shunted by a resistance. Referring to the diagram, Fig. 1, for the meaning of the symbols, if V is the instantaneous potential across the condenser,

$$V = I_2 R \qquad I_1 = j\omega c V \qquad I = I_1 + I_2 = V\left[\frac{1}{R} + j\omega c\right]$$

$$I^2 = V^2\left[\frac{1}{R^2} + \omega^2 c^2\right],$$

and there is a phase difference between I and V given by

$$Cos\ \theta = \frac{1}{[1+\omega^2 c^2 R^2]^{\frac{1}{2}}}.$$

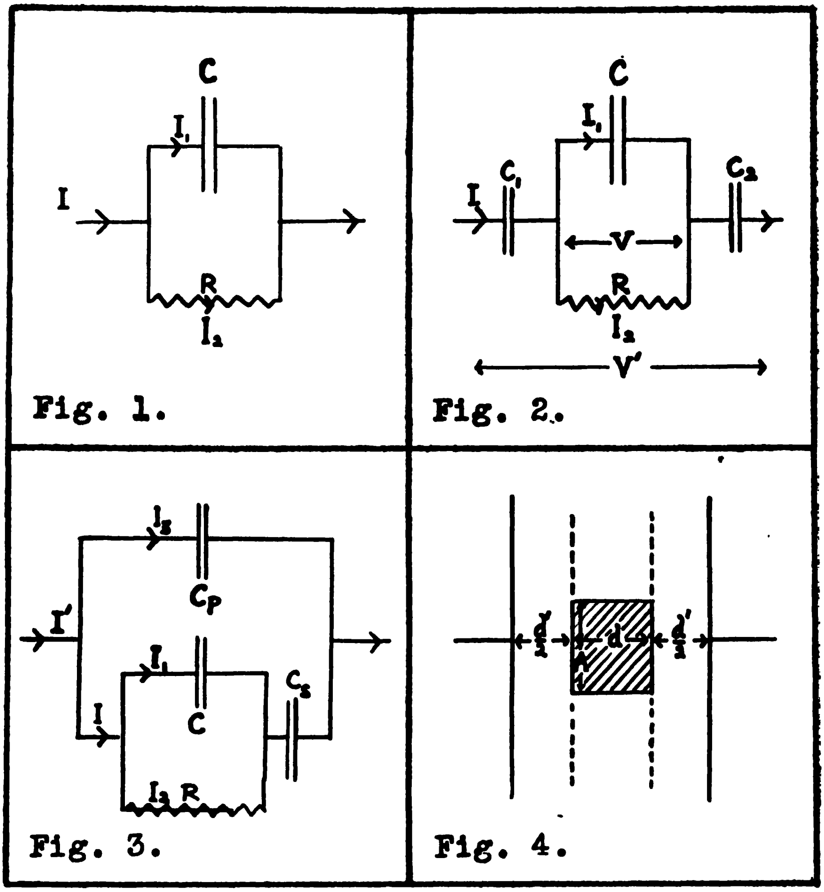


FIG. 1, 2, 3 and 4. *'Equivalent circuits'.*

Power loss
$$P = VI \cos\theta = \frac{V^2}{R}, \tag{3}$$
or
$$P = I^2 \cdot \frac{\frac{1}{R}}{\left[\frac{1}{R^2} + \omega^2 c^2\right]} = I^2 \cdot \frac{R}{1+\omega^2 c^2 R^2} \,. \tag{4}$$

In any experiment, we may arrange, as we alter the value of R, by, for instance, replacing the solution in the condenser by another of different conductivity, that either the potential V or the total current I is kept constant. In the first case, V being constant, the maximum heating is when R is least. In the second case, differentiating in (4) we get for the maximum heating the relation,
$$R = \frac{1}{\omega C} \,. \tag{5}$$
The latter is one of the cases dealt with by Pätzold.

(b) Suppose now that the circuit is as in Fig. 2, capacities C_1 and C_2 being added in series with the "leaky" condenser. This is the equivalent circuit of the case where the electrodes are not in contact with the solution

but placed outside the cell containing it. Let V' now be the total drop of potential between the condenser plates of the oscillator.

As in (a)

$$V = \frac{I}{\frac{1}{R} + j\omega c}.$$

There is a potential drop across the condenser C_1 given by $V_1 = \frac{I}{j\omega c_1}$, and across C_2 given by $V_2 = \frac{I}{j\omega c_2}$.

Then $V' = V_1 + V_2 + V = I\left[\frac{1}{j\omega}\left(\frac{1}{c_1} + \frac{1}{c_2}\right) + \frac{1}{\frac{1}{R} + j\omega c}\right].$

Let $\frac{1}{c_1} + \frac{1}{c_2} = \frac{1}{c_s}$, c_s being the total capacity in series with the "leaky" condenser.

Then

$$I = \frac{V'}{\frac{1}{j\omega c_s} + \frac{1}{\frac{1}{R} + j\omega c}}.$$

Rationalizing, and making use of the convenient abbreviations $\frac{c_s}{c} = a$ and $\frac{1}{R\omega c} = h$, we find that the real component of I, that is, the component in phase with V, is:—

$$I_R = \frac{V'\omega c h}{(1+a)^2 + a^2h^2},$$

and the power loss is therefore:—

$$P = V'I_R = \frac{(V')^2\omega c h}{(1+a)^2 + a^2h^2}$$

$$= (V')^2 \frac{R}{R^2\left(1+\frac{c}{c_s}\right)^2 + \frac{1}{\omega^2 c_s^2}}. \tag{6}$$

Since there is no added loss in the capacity C_s, the power loss in terms of the current I is, as before,

$$P = I^2 \frac{R}{1+\omega^2c^2R^2}.$$

Examination of these equations as R varies shows that there are now maxima in each of the two cases, that of constant voltage and that of constant current. The values of R for maximum heating are respectively

$$\text{Constant voltage:} - \quad R = \frac{1}{\omega(c+c_s)}. \tag{7}$$

$$\text{Constant current:} - \quad R = \frac{1}{\omega c}, \text{ as before.}$$

(c) If the area of the condenser plates is greater than the cross section of the liquid in the cell, there will be in addition to the capacities already considered, a capacity C_p between the condenser plates in parallel with the circuit of (b). There is an additional current through C_p,

$$I_1 = j\omega C_p V'.$$

There is, however, no additional power loss here, so that the total heating is still given by (6),

$$P = (V')^2 \frac{R}{R^2\left(1+\frac{c}{c_s}\right)^2 + \frac{1}{\omega^2 c_s^2}} .$$

The relation between the total current I' and the potential is now changed.

$$I' = I + I_1 = V'\left[\frac{1}{\frac{1}{j\omega c_s} + \frac{1}{\frac{1}{R}+j\omega c}} + \frac{1}{\frac{1}{j\omega c_p}}\right].$$

This gives for the power loss:—

$$P = (I')^2 \frac{R}{\left(1+\frac{c_p}{c_s}\right)^2 + \omega^2 R^2\left[c\left(1+\frac{c_p}{c_s}\right)+c_p\right]^2} . \tag{8}$$

The conditions for maximum heating are then:—

$$\text{Constant voltage:} - \quad R = \frac{1}{\omega(c+c_s)}, \text{ as in (7).}$$

$$\text{Constant current:} - \quad R = \frac{1}{\omega\left(c+\frac{c_p c_s}{c_p+c_s}\right)} . \tag{9}$$

In our experiments, the voltage was constant, and Equation (7) applied; in Pätzold's experiments the total current I' was kept constant and Equation (9) was applicable.

In either case, if the ratio $\frac{c_s}{c}$ is small compared to unity, the formula for the power loss becomes,

$$P = V^2 c_s^2 \frac{\omega^2 R}{1+\omega^2 c^2 R^2} = (I')^2 \left(\frac{c_s}{c_p+c_s}\right)^2 \frac{R}{1+\omega^2 c^2 R^2}, \tag{10}$$

and whether current or voltage is regarded as constant, the maximum is when $R = \frac{1}{\omega c}$ approximately. This was the law proved experimentally to hold for aqueous solutions over a range of wave-lengths from 10 to 200 metres. In each case, when $\frac{c_s}{c}$ becomes appreciable in magnitude, the effect is to increase the resistance that gives a maximum production of heat. The existence of this "capacity shift" is proved experimentally in a later part of this paper.

The conditions of practical radiothermy differ from those of the experiments in an important particular. In the latter we find the maximum heating by replacing a particular solution with its values of R and C, by solutions of different conductivity, whilst in the practical case, all the different substances are present simultaneously in the field, and we wish to know in which of them most heat is developed.

Suppose that we have a number or circuits like that of (c) arranged in parallel. Then for all of them the voltage V' is the same, and the power loss in each of them is given by the formula (6). Let us suppose that the nth circuit, say, consists of a volume of material, of cross section A, at right angles to the field, and length d, and that the total distance between its faces and the oscillator plates is d^1. Let its dielectric constant and conductivity be K and x

respectively. Then, see Fig. 4,

$$c=\frac{KA}{4\pi d},\quad R=\frac{d}{Ax},\quad c_s=\frac{A}{4\pi d^1}.$$

Substituting in (6) and putting $\omega=2\pi\nu$, ν being the frequency, we find:—

$$P=\frac{(V')^2}{(d^1)^2}(A.d)\frac{\nu^2 x}{\nu^2\left(K+\frac{d}{d^1}\right)^2+4x^2}.$$

Or dividing by the volume, (Ad), the heat per unit volume produced per second

$$H=\frac{(V')^2}{(d^1)^2}\frac{\nu^2 x}{\nu^2\left(K+\frac{d}{d^1}\right)^2+4x^2}. \tag{11}$$

If $\frac{d}{d^1}$ is small compared to K, this becomes

$$H=\frac{(V')^2}{(d^1)^2}\frac{\nu^2 x}{\nu^2K^2+4x^2}. \tag{12}$$

Let the circuits now be in series. In this case, the total current I' is the same for each of them, and the power loss in any one of them is given by (8). If the same condition, that $\frac{c_s}{c}$, which equals $\frac{d}{Kd^1}$, is small compared to unity, holds, this reduces to (10), *i.e.*,

$$P=(I')^2\left(\frac{c_s}{c_s+c_p}\right)^2\frac{R}{1+\omega^2R^2c^2}.$$

Substituting for R and C in terms of the dielectric constant, K, and the conductivity, x, we get,

$$P=(I')^2\,4\frac{d}{A}\left(\frac{c_s}{c_s+c_p}\right)^2\frac{x}{\nu^2K^2+4x^2},$$

and dividing by the volume, we find, for the heat per unit volume per second

$$H=4\left(\frac{I'}{A}\right)^2\left(\frac{c_s}{c_p+c_s}\right)^2\frac{x}{\nu^2K^2+4x^2}. \tag{13}$$

Equations (12) and (13) show, on differentiation, that, even in the complex equivalent circuit of parallel and series combinations that represents the conditions of practice, the law of maximum heating is approximately (for constant K)

$$x=\frac{\nu K}{2}. \tag{14}$$

In fact, the heat produced per second per unit volume of any part of the body, where the dielectric constant is K and conductivity x, is given by

$$H=V^2f\cdot\frac{\nu^2 x}{\nu^2K^2+4x^2}, \tag{15}$$

where f is a function determined by the shape, position, electrical properties of the portion considered and its neighbors, *but is not dependent upon the frequency.*

The condition that must hold for this to be approximately true is, as has been shown, that $\frac{c_s}{c}<<1$ or that $\frac{d}{d^1}$ is small compared to K. The dielectric constant of the majority of body substances is of the order of magnitude of that of water, *i.e.*, 80, for the frequencies employed in practice. We have an approximation to 10% then if $d^1>\frac{d}{8}$, *i.e.*, if the distance of the body from the

plate of the oscillator is greater than ⅙th of the width of the body, *i.e.*, if it is greater than about one inch. In the usual conditions of medical practice this condition is satisfied. It must be noted, however, that in the case of diathermy with electrodes in contact with the body, the formula has no application, the problem being a much more complex one. In this case, if the various bodies are considered to be in series with each other, Equation (4) applies and there is a selection of the substance obeying relation (5); but if on the other hand, they are considered to be in parallel, Equation (3) shows that the best conductor is the most heated. Any actual case must be a complex combination of the two limiting cases. We can say, however, that increasing the frequency tends to favor in general the heating of the better conductor.

The Equation (15) for the heating can be deduced, quite simply if not rigorously, from the general equations of the electromagnetic field. The differential equation that must be satisfied at every point in the field is that deduced from Maxwell's equations for a conducting medium,

$$\frac{d^2R}{dt^2}+\frac{4\pi x}{K}\,\frac{dR}{dt}=\frac{1}{\mu K}\nabla^2 R, \tag{16}$$

together with the boundary conditions, that at every surface of separation of two media the quantity $\left(\frac{K}{4\pi}\,\frac{dR}{dt}+xR\right)$ is continuous along the normal to the surface. The condition that at the condenser plates the potential is sinusoidal then completes the description of the problem. In the solution of these equations for the case of the Hertzian oscillator, which closely resembles the present problem, it is shown that at distances small compared to the wave-length of the oscillator, the "induced", electromagnetic, contribution to the total electric field is negligible, and the field is approximately entirely "electrostatic". That such is the case for the field between the plates of a high frequency oscillator has been shown experimentally by Pariseau (8). This means, that within any region that is homogeneous, the distribution of the field, though dependent upon the properties of the medium there and upon its shape, etc., is independent of the frequency. (To say that "skin effect" is negligible is an equivalent statement.)

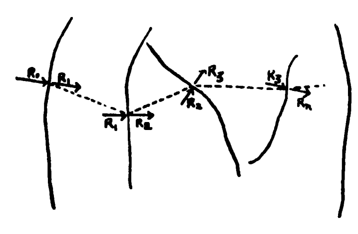


FIG. 5. *Electric field in body.*

The heterogeneous body may be considered to consist of a number of regions in which the properties are homogeneous, separated by "surfaces of separation". Within one of these regions we can find two points on the surface of separation where the normal component of the field, within the region, is the same. Let these points (as in Fig. 5) be joined by a line which is produced so that this is true for each region through which it passes. Then at the intersections of the line with the surfaces of separation of the regions, we have the equations for the normal induction:—

$$\frac{K_1}{4\pi}\,\frac{dR_1}{dt}+x_1R_1=\frac{K_2}{4\pi}\,\frac{dR_2}{dt}+x_2R_2=\dots\dots\dots\dots=\frac{1}{4\pi}\,\frac{dR_0}{dt}, \tag{17}$$

where R_0 is the normal intensity at the point of emergence of the line from the body to the air. Since the solutions for R_n must contain the time factor $e^{j\omega t}$, the equations become:—

$$R_1\left[\frac{K_1}{4\pi}\,j\omega+x_1\right]=R_2\left[\frac{K_2}{4\pi}\,j\omega+x_2\right]=\;.\;.\;.\;.\;.\;.\;.\;.\;.\;.\;.\;.\;.\;=R_0\,\frac{j\omega}{4\pi}$$

or

$$R_n=R_0\,\frac{j\omega}{K_n j\omega+4\pi x_n},$$

which gives, putting $\omega=2\pi\nu$,

$$R_n^2=R_0^2\,\frac{\nu^2}{\nu^2K_n^2+4x_n^2}\,. \tag{18}$$

The average intensity $\overline{R_n}$ within the region (n) will be connected with the intensity R_n at the point of intersection of the line with the surface, by a relation that, as has been shown, does not involve the frequency ν. R_0 will be proportional to the potential difference between the plates of the oscillator, the factor of proportionality again being independent of the frequency. We have then the final equation

$$\overline{R}_n{}^2=V^2f\,\frac{\nu^2}{\nu^2K_n{}^2+4x_n{}^2},$$

where f does not depend on ν. The heat produced per second per unit volume is, by a simple calculation, shown to be equal to $\overline{R_n^2}x$, and therefore we have,

$$H_n=V^2f\,\frac{\nu^2x_n}{\nu^2K_n{}^2+4x_n{}^2},$$

which is identical with Equation (15).

In any practical case, the prediction of the absolute values of the rise in temperature of the various parts of a complex heterogeneous body is impossible, because of the complexity of the functions that involve the distribution of the electric field throughout the body. The formula does, however, give us very useful information. It indicates, that, if we wish to *favor* the heating of any particular region, we must choose the frequency ν of the oscillations so that the quantity $\frac{\nu^2x}{\nu^2K^2+x^2}$ is greater for that region than for other regions.

Fig. 6 shows how this quantity varies with increasing frequency, assuming different values for the constants K and x. Curve 1 corresponds closely to that of blood,

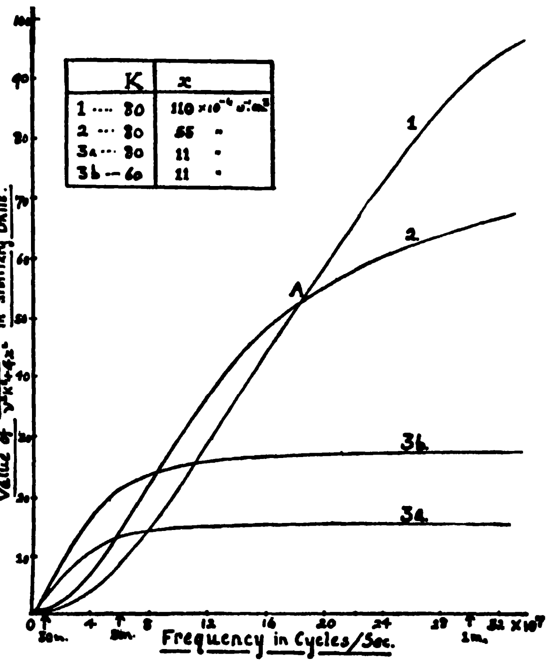


FIG. 6. *Graph showing change of relative heating of different substances with change of frequency.*

whilst 2 is for a substance of the same dielectric constant but having a conductivity of half the value. Curve 3*a* is for a substance of conductivity one-tenth that of blood, and corresponds to the curve for the proteins of the blood. The effect upon the curves of changing the value of K is seen by comparing 3*b* with 3*a*.

A practical application of such curves suggests itself as follows. Suppose that we wish to heat certain diseased cells in the body, without injury to the neighboring normal cells. Let the electrical constants of the normal cells be K, x, whilst those of the diseased cells are found to be K', x'. The curves with these parameters will intersect at some point such as A in Fig. 6. This may be described as a "point of inversion" for the two substances, for frequencies less than that of this point favor the heating of the one substance; frequencies greater than this favor the heating of the other. There is a very simple expression giving the frequency at which this inversion occurs. It is

$$\nu_c^2 = \frac{4xx'(x'-x)}{K^2x'-(K')^2x}, \tag{19}$$

which, in the special case of $K=K^1$ reduces to

$$\nu_c = \frac{2}{K}\sqrt{xx'} . \tag{20}$$

Frequencies less than ν_c will favor the heating of the substance for which the ratio $\frac{x}{K^2}$ is the lesser, frequencies greater than ν_c favor the heating of the other. (No point of inversion exists if $\frac{(x'-x)}{K^2x'-(K')^2x}$ is negative, in which case all frequencies favor the substance of the greater $\frac{x}{K^2}$.) From the values of the dielectric constants and conductivities of the diseased and of the normal cells we can thus deduce the frequency best suited to the end in view. More complete knowledge of the electrical properties of body substances for high frequencies would here appear to be desirable.

In the special case where the dielectric constant is assumed to be the same for all the substances, the favored substance is the one for which the relation, $x = \frac{K\nu}{2}$, is true. The conductivity in this equation is in absolute electrostatic units. A convenient practical form may be given to the relation which will apply to dilute aqueous solutions only. If λ be the wave-length of the oscillations in air expressed in metres, the conductivity of the solution that is most heated is

$$x = \frac{130}{\lambda} \times 10^{-4}\omega^{-1},$$

or if the frequency be N million cycles,

$$x = \frac{4}{9} N \times 10^{-4}\omega^{-1}.$$

From tables of conductivities the corresponding concentration can be found, which, for instance, would be the most suitable as a medium for biological preparations which it was desired to heat by the short waves.

Search is still being made, by workers in the biological and biochemical field,

for specific effects of the waves upon living cells other than those traceable directly to the heating effect. No definite evidence of such has yet been found. Apparent specific effects might be produced by a selective heating of particular cells greater than that which would be produced by a general heating of their surroundings by ordinary means. In the case of laboratory experiments, the foregoing equations provide a method of discrimination between the heating effect and any other effects of the waves that may be present. For by variation of the conductivity of the media used in the experiments, keeping other conditions the same, the amount of heat produced can be varied over a wide range, without seriously modifying any other specific effect of the waves.

Experimental Work

The shortest wave-length used in the previous experiments was 11.5 metres. In order to reach shorter wave-lengths a new oscillator had to be constructed and the technique of the measurements considerably altered. Its construction follows closely that described by Gill and Morrell (3, 4). The plate and grid inductances are simply two parallel brass tubes, supported horizontally about 10 cm. apart. They slide telescopically into other tubes, so that their total length can be adjusted from one to three metres. At one end they are connected to the grid and plate of a U.X.852 tube, and at the other they are bridged by a variable air condenser of high voltage insulation, of maximum capacity 50 microfarads. Here connections are made through air-spaced chokes to the d.-c. supply on the one side and to the grid-leak and earth on the other. With a plate voltage of about 1000 volts, and using a grid-leak of 25,000 ohms, the set will give oscillations of wave-length from 3 to 8 metres. The design is a convenient one, as by interchange of the usual voltages on the grid and plate the electron oscillations of Barkhausen and Kurtz are produced.

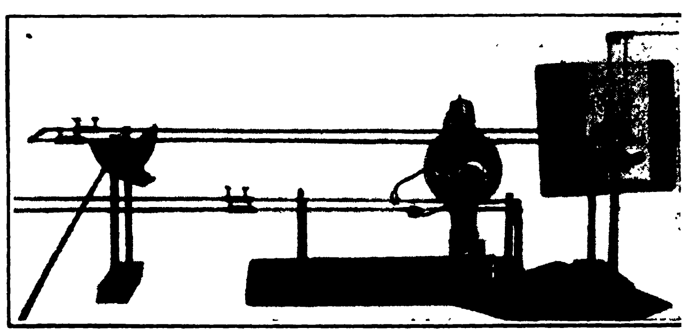


FIG. 7. *Arrangement of apparatus for experiments with 6.5-metre waves.*

With such high frequencies, it was quite impracticable to have the substance under test heated directly in the condenser of the oscillator, as its presence profoundly affected the output of the latter. A secondary circuit was therefore used, consisting of a variable frame of parallel rods, closed at one end and carrying the heater condenser plates at the other. This frame was supported just above the rods of the oscillator, and in each experiment, with the body to be heated in position, its length was adjusted until there was resonance with the oscillator beneath. Thus, although the body in the test condenser was changed, the frequency was kept the same throughout. The field in the

secondary condenser, however, was not constant, and a comparison method had therefore to be used. Two solutions, one a standard, the other varied, were held in two small sausage-shaped Pyrex vessels, provided with funnels for filling and outlets for draining, which were supported side by side in the condenser with their axes parallel to the field. Temperatures were taken before and after heating by two copper-constantan junctions, held in an ebonite block, which when lowered, brought the junctions into the centre of the flasks. The photograph, Fig. 7, shows the details of construction.

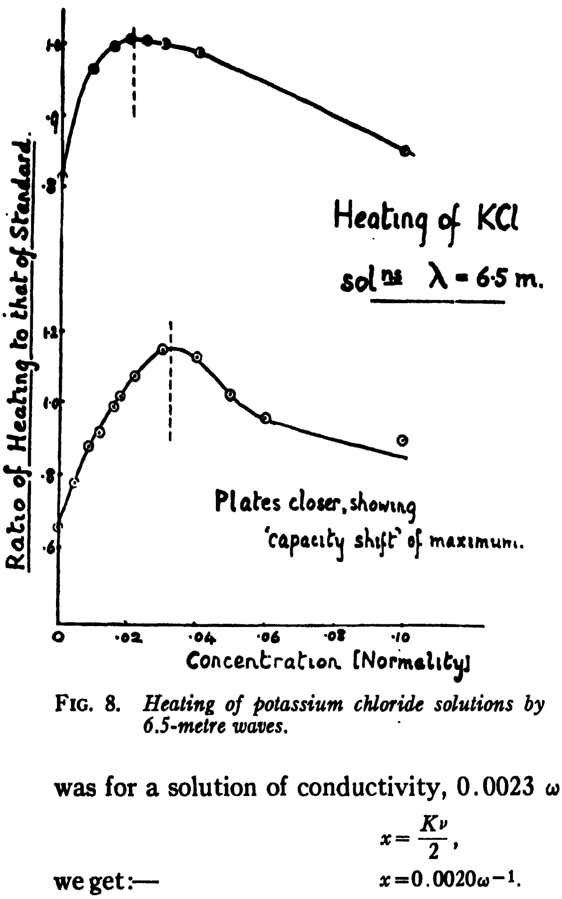


FIG. 8. *Heating of potassium chloride solutions by 6.5-metre waves.*

With this apparatus, the rise in temperature of different solutions of potassium chloride was compared with that of the standard solution, (0.016N) so chosen that its heating was of the order of the average of those of the range of solutions used. The results are shown in Fig. 8. In each case, the solutions were interchanged in the two flasks, and the average result taken, to eliminate the inequalities in the heatings of the latter themselves. The wave-length, measured on a Lecher wire system, was 6.5 metres. The maximum found was for a solution of conductivity, 0.0023 ω^{-1} while, using the relation

$$x = \frac{K\nu}{2},$$

we get:—

$$x = 0.0020\omega^{-1}.$$

Thus, the law is valid within the experimental error at this higher frequency. We would expect a departure only when we reach a region of "anomalous dispersion", where the dielectric constant changes. It is very unlikely that for the substances of the body this region is reached until the wave-length is less than one or two metres. It does not seem possible that wave-lengths of less than, say, five metres can be applied to medicine, as the capacity introduced by the body would make higher frequencies unattainable by all the presently known methods of production of such oscillations. The dielectric properties of body substances must be more completely examined, but for practical purposes the law of maximum heating enunciated is of application.

The modification introduced into the law when we pass from the "ideal" case to the practical has been discussed in the theoretical section that precedes.

Its existence was shown experimentally by the apparatus just described. The plates of the condenser in the secondary circuit were brought closer together, so that the capacity between them and the flasks was increased, and the curve of the heating against concentration was again determined. The lower curve of the figure shows how the maximum was shifted to a higher value of the concentration, the shift amounting to about 50%. From Equations (7) and (9) it has been shown that this "capacity shift" is approximately represented by the correcting factor $\left(1+\frac{c_s}{c}\right)$. The value of this term was estimated by the use of a heterodyne device for the measurement of the capacities involved. Two oscillators of wave-length about 25 metres were made to give an audible "beat" note by their mutual action. The frequency of one was fixed; that of the other was controlled by the value of the capacity, either of a standard calibrated variable condenser or by the throw of a switch, of the condenser to be measured. The variable condenser was adjusted until the constancy of the beat note, when the switch was thrown from one side to the other, indicated that it was equal to the unknown capacity. Owing to the smallness of the capacities and to the difficulties involved in the measurements at such high frequencies the results were not of great accuracy, but indicated that the shift was of the order of magnitude predicted by the formula.

Experiments Demonstrating the Selective Heating

In order to demonstrate that, in cases approximating to those of practical diathermy, the selective effect exists and is modified by the choice of wave-length, some qualitative experiments were carried out with substances of biological interest.

A substance that is heat sensitive, namely, tetriodomercurate of silver, had been of service in previous experiments (7). It has the property of changing reversibly from a yellow to a red color at about 35 to 40° C. By pulverizing some of the dry salt and intimately mixing it with turpentine and a little glycerine, added to prevent settling, a paint was prepared that showed the same heat sensitivity as the original substance. The change of color may readily be recorded photographically if mercury green light, from a mercury arc filtered by a suitable filter, be used as illuminant with a panchromatic plate in the camera. Exposures of 15 sec. only were found necessary in our experiments.

Slices of meat, about ½ in. thick, that contained representative substances such as fat, muscle, bone, etc., were first photographed so that the various parts could afterwards be identified. Their surfaces were then covered completely with the yellow paint, and photographed again to show the uniformity of color. The meat was supported on a glass plate between the condenser plates of the oscillator, and the development of heat in the various parts could be followed by taking photographs at successive intervals. Results are shown in Fig. 9, 10 and 11. In Fig. 9 the meat is a pork chop. Two hot spots became evident almost immediately as can be seen in the photographs. When examined afterwards, there were found at these places two small blood vessels embedded

in fat. They are just visible in the photograph of the unpainted meat. In general, it was noted that the fat portions might be raised to a very high temperature—sufficient to cause 'frying'—while the lean portions had been heated comparatively very little. The fact that the specific heat of the fat is much less may partly explain this. The very white, outside, fat remained however comparatively cold. There is apparently a tendency for more heat to be developed at the 'corners' of the specimen due to the distribution of the electric field here.

The piece of beef in Fig. 10 shows similar characteristics of the heating—but here most heat was developed in the 'connective tissue' between two bones—(which latter seemed to heat least of all). Fig. 11 demonstrates clearly that the localization of heat is not determined solely by the concentration of electrical resistances and of specific heat in various parts, but is also due to a definite selective action of the waves. For here the same piece of meat (pork) has been heated both by 25-metre waves and by 10-metre waves and the course of the heating followed in each case. The change of color starts at one spot with the 25-metre wave and spreads to the fat portion to the right hand side in the photograph. Whilst with the 10-metre waves, the red color appears simultaneously in two places and subsequent spreading proceeds in a different manner. The condensers in which the experiments were made were geometrically similar, and to eliminate the effect of permanent changes in the meat produced by the heatings, these were made alternately, *i.e.*, first for a short period with 25 m., then for a short period with 10 m.; then for a longer time with 25 m., and finally for the longer time with 10 m. The progressive changes were repeated in each case, and there is no doubt that the change in wavelength had changed the distribution of the heating.

For use in further experiments, a thermocouple needle was constructed from a No. 22 gauge hypodermic needle, of length 2 in. An insulated No. 40 gauge constantan wire runs down the inside of the needle and forms a junction with the steel needle at the oblique orifice at its point, this orifice being closed with a touch of solder. The needle is mounted on a bakelite tube in which the cold junctions lie side by side, a steel wire of small diameter being brazed to the top of the needle so that the junction of dissimilar metals may be made inside the tube. 'Telephone' leads are the connections to the Cambridge potentiometer, which, with a Kipp and Zonen galvanometer, has a sensitivity sufficient to give readings of the temperature of the point of the needle quickly and accurately to $\frac{1}{50}$° C. The construction of such and of more elaborate thermocouple needles is described by Karrer and Estabrook (5). For more accurate work the cold junctions should be kept at constant temperature—in an ice bath—but for comparative measurements the needle described is more convenient.

With its aid, a study was made of the heating of a hen's egg by the short waves. The shape of an egg is such that the distribution of the electric field in it is more simple than for other shapes—approximating to a uniform field, if the interior were of homogeneous dielectric properties. Moreover, search is still being made by workers in this field for specific effects, other than those

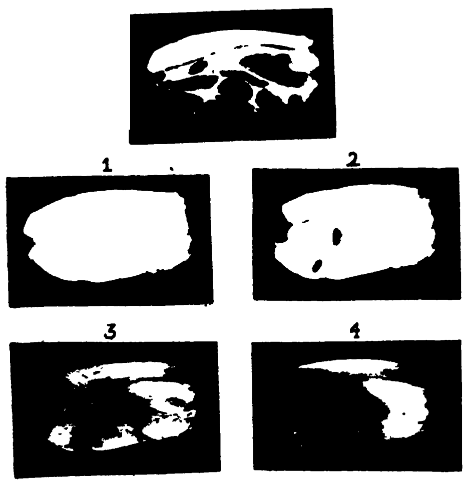


Fig. 9. *Heating of meat (pork) by 25-metre waves.*

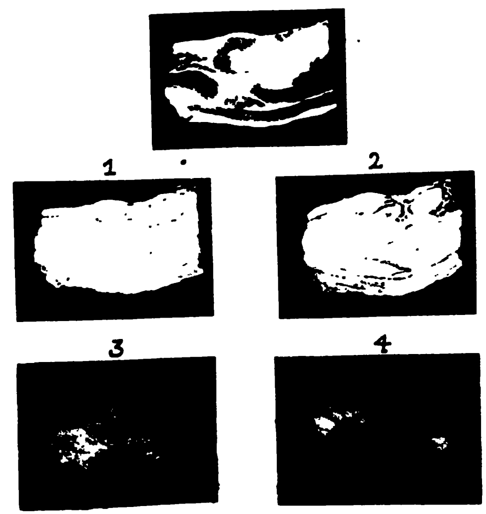


Fig. 10. *Heating of meat (beef) by 25-metre waves.*

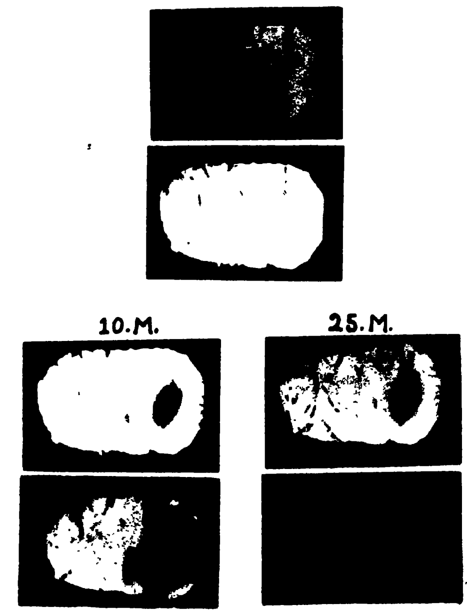


Fig. 11. *Heating of meat by 10-metre and by 25-metre waves.*

due to heating, of the short waves on living organisms, and attempts have been made to modify the development of the embryo in eggs. An enquiry into the physical process of heating of the interior of an egg might then be of service.

The eggs were hung in the field of the oscillator between the plates in a cradle of threads, with the long axis of the egg parallel to the electric field. The thermocouple needle was clamped vertically to an adjustable stand such that it could be racked up and down and the temperature at different points across a vertical diameter of the egg could be measured by insertion of the needle through a small hole in the shell. Initial measurements having shown that the interior was at uniform temperature, the egg was subjected to the oscillations for a given period. After heating, the egg was removed and the temperatures across the diameter again taken. If the interior of the egg were entirely homogeneous we should have a practically homogeneous electric field throughout, and would find that the resultant distribution was almost uniform, though cooling from the outside would make the temperature higher in the middle.

Results are shown graphically in Fig. 12. With the 25-metre waves there is distinct evidence that the temperature rise is greater in the white than in the yolk. The measurements must be given a qualitative significance only, as errors due to cooling, unequal conduction down the needle in the different positions and to other factors that are unavoidably present. The minimum temperature in the centre was, however, found repeatedly. When a wave-length of 10 metres was used, however, the eggs in no case showed this minimum, but in many cases a small increase in temperature at the centre, indicating that here the yolk heated as much if not more than the white. With the oscillator giving waves of 200 metres, there was evidence of the selective heating of the white as with 25 metres, but sufficient heat could not be produced with the equipment available to give reliable results.

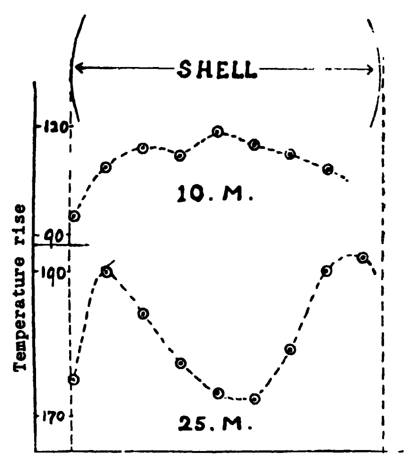


FIG. 12. *Rise in temperature of the interior of an egg heated by short waves.*

The white and yolk were then separated and heated together in the field under the same conditions. They were placed in two similar small test tubes hung side by side with their axes parallel to the field between the condenser plates. The rise in temperature produced by the oscillations was taken by a mercury-in-glass thermometer. Heating was very rapid indeed compared to that produced in the eggs indicating how great is the shielding effect of the shell. Results at the different frequencies are shown in Table I.

In the case of the 10-metre heating, the substances were interchanged in their test tubes and experiments repeated to eliminate effects due to unequal

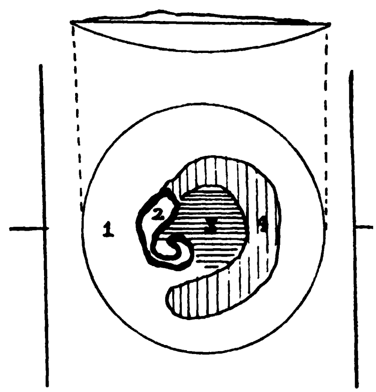
TABLE I

	10 Metres			25 Metres			190 Metres		
	Initial Temp., °C.	Final Temp., °C.	Difference, °C.	Initial Temp., °C.	Final Temp., °C.	Difference, °C.	Initial Temp., °C.	Final Temp., °C.	Difference, °C.
White	25.0	36.7	11.7	27.0	42.0	15	24.5	28.3	3.8
Yolk	25.5	43.5	18.0	27.0	48.0	21	25.0	29.0	4.0
Ratio: $\frac{\text{White}}{\text{Yolk}}$	0.65			0.70			1.05		

heating of the latter themselves. The difference amounted to 5%. For comparison double distilled water, and 0.002 N KCl solution were heated in the test tube by the 25-metre waves with the same time as above. The rises in temperature were 6° and 40° C. respectively. The conclusion is that under the same conditions of external field, shape and position, there is selective heating of the yolk at 25 metres; the ratio is increased at shorter wave-lengths; but at 200 metres the white heats more than the yolk. The specific heat of the yolk is less than that of the white, and this plays its part, but, of course, in the same way for each of the wave-lengths, so that by experiments at different frequencies we differentiate a true 'selective' effect from that due to specific heat differences. Yet in the egg we found greater heating of the white than of the yolk at 25 and at 200 metres, though the yolk has the advantage of lying nearer to the maximum for a given field. Thus the field induced in the white must be greater than that in the yolk. This is what we would predict from the difference in the dielectric constants of the two substances. Blüh (1) gives for a wave-length of 50 cm. the values—white 68.0, yolk 60.0. We have shown, in our previous paper, that whether the dielectric constant of the outer substance be greater or less than that of the inner, its effect is to shield the latter from the field; and if for our frequency the dielectric constant of the white be the greater, the concentration of lines of force would be the greater there. The heating of the egg is an excellent example of how the various factors, position, dielectric constant, conductivity, and wave-length play their parts.

To complete the investigation, some experiments were made with fertilized eggs. It was hoped that, by 'chandling', the position of the embryo could be seen, and after heating the egg in the field the temperature of the embryo could be taken with the thermocouple needle. This was not found practicable, so the shell was broken and the contents placed in a watch glass which was supported in the centre of the condenser field. After heating by the oscillations the temperature of the embryo and of the surrounding fluids could be found by the thermocouple. The diagram shows the relative positions of the contents

of the egg, and gives the relative rise in temperature of the parts after heating. Temperatures were taken in the order shown and then in the reverse order, to eliminate difference due to cooling. The measurements were repeated with an embryo that showed less development. The results in general are that the greatest rise in temperature is produced in the allantoic excretion — next of about equal amount in the area *opaca vasculosa* or yolk-sac, and in the body of the embryo, and least in the surrounding white. With 10 metres the relative heatings were about the same, with the relative heating of the body of the embryo increased.



Part	Rise in temp.
1. White	4.5°
2. Body	8.5°
3. Excretion	10.0°
4. Yolk-sac	9.5°

FIG. 13. *Rise in temperature of a fertilized egg heated by short waves.*

There is some evidence from experiments *in viva* on the heating of animals in the field of an oscillator of 30 metres wave-length, that the liver rises in temperature more than the heart (2). Experiments were made to find the relative rises in temperature of these media, *in vitro*, when under the same conditions in the field. The substances (calves' liver and heart) were packed into the two test tubes previously described and heated side by side in the field. Wave-lengths of 10, 25 and 200 metres were used, care being taken that the arrangement of the test tubes between the condenser plates was geometrically the same in the three cases. The results, given in the Table II, are very interesting.

TABLE II

	25 Metres				200 Metres				10 Metres			
	Initial Temp., °C.	Final Temp., °C.	Rise	Ratio	Initial Temp., °C.	Final Temp., °C.	Rise	Ratio	Initial Temp., °C.	Final Temp., °C.	Rise	Ratio
Liver	21	28	7.0	1.55	20	26.5	6.5	1.85	26.0	43.5	17.5	0.8
Heart	21	25.5	4.5	1	20	23.5	3.5	1	25.0	46.0	21.0	1

It will be seen that though at 25 metres the heating of the liver is favored, at shorter wave-lengths there is reversal. The results of experiments conducted *in vitro* cannot be transferred without caution to conditions *in viva*, but it may safely be concluded that if for any reason it were desired to enhance this selective heating of the liver in radiothermy, the longer wave-lengths would be more effective; and that at shorter wave-lengths the relative heating of the heart would become of more importance.

The results have, therefore, demonstrated the existence of the selective effect and its control by choice of wave-length. Whether or not the phenomena have any practical importance in the medical application, depends on whether the blood flow and rapid interchange of heat in the living body render the differences of temperature that the oscillations would undoubtedly produce, negligible or not. Experiments *in viva* upon animals will alone settle the point. There is already some evidence which has been referred to, in the heating of the heart and of the liver.

Summary

The theoretical basis of the formula previously given for the heat produced in a poorly conducting dielectric, which is placed in the field of a high frequency oscillator, has been examined in detail. Its application has been made to the complex practical case met with in medical radiothermy. Prediction of the selective heating effect is possible from a knowledge of the characteristic electric constants of the substances of the body, and by suitable choice of wave-length the heating of a particular part of a heterogeneous body may be favored over that of neighboring regions. The analysis applies with a satisfactory degree of approximation to the case of radiothermy, but not to electrode diathermy.

The previous experiments have been extended to shorter wave-lengths, and experiments on the heating of meat have demonstrated the selective action and its dependence upon wave-length. The longer wave-lengths favor the heating of the heart, shorter wave-lengths that of the liver. The rise in temperature of various parts of an egg has been measured as an example of the various factors that are involved.

References

1. BLÜH, O. Protoplasma, 3: 88. 1927.
2. CARPENTER., C. M. Arch. Phys. Therapy, X-ray and Radium. v. 12. no. 3, p. 141. March, 1931.
3. GILL, E. W. and MORRELL, J. H. Phil. Mag. 44: 161-178. 1922.
4. GILL, E. W. and MORRELL, J. H. Phil. Mag. 49: 369-379. 1925.
5. KARRER, S. and ESTABROOK, G. B. J. Maryland Acad. Sci. 1: 129-147. 1930.
6. McLENNAN, J. C. and BURTON, A. C. Can. J. Research, 3: 224-240. 1930.
7. McLENNAN, J. C. and BURTON, A. C. Nature, 126: 130. 1930.
8. PARISEAU, L. E. Can. Med. Assocn. J. 20: 146-152. 1929.
9. PÄTZOLD, J. Zeits. fur Hoch-Frequence Technik, 36: 85-98. 1930.
10. PIERCE, G. W. (Unpublished work, referred to by Richards, W. T. and Loomis, A. L. Proc. Nat. Acad. Sci. 15: 587-593. 1929.)

THE RANGE OF THE ALPHA-PARTICLES FROM URANIUM II[1]

By S. Bateson[2]

Abstract

The range of the α-particles from uranium II has been determined by a scintillation method to be 3.29 ± 0.08 cm. at 15° C. and 760 mm. This is in good agreement with Laurence's value found with a Wilson chamber. From the Geiger-Nuttall relationship the period is calculated to be 28,000 years, a value considerably less than that found recently by direct measurement.

Introduction

The range of uranium II was measured by Geiger and Nuttall (5) in 1912 using an ionization method. Their value of 2.9 cm. at 15° C. and 760 mm. was later corrected by Geiger (4) to 3.07 cm. In 1924 Gudden (6) calculated the range to be 2.91 cm. by measuring pleochroic haloes in fluorite. More recently Ziegert (15) has obtained the total ionization in air for several radioactive substances including uranium II. Calculation using the total ionization as given by Ziegert gives 3.13 cm. for the range of uranium II. Meanwhile Rutherford (11) had estimated that the range was probably not less than 3.23 cm. The most reliable determination of the range of uranium II was made by Laurence (7) who obtained a value of 3.28 ± 0.03 cm. using a Wilson chamber. In view of the wide variation in these values, it seemed advisable to check the range by a different method.

Method

The scintillation method was adopted as the most feasible. This method has been used successfully to determine ranges by Rothensteiner (10), Taylor (13), and more recently by Philipp (9).

The principal difficulty of the scintillation method lay in the low activity of uranium II. Its practicability depends on whether or not a sufficient number of particles can be counted to insure reasonable accuracy. The effect of using a thick layer of active material is to increase the straggling of the α-particles, making the range harder to determine as well as shortening its value. Since the source had to be thin its area was increased by placing it at the centre of curvature of a glass segment on which the active material was deposited. In this way variations in the distance from source to screen for various portions of the source were almost entirely eliminated.

A preliminary calculation was made of the total number of scintillations per minute to be expected from a layer of material having a thickness equivalent to 1 mm. of air. This value was approximately one scintillation per minute. It was decided that this number, although small, was sufficiently large to obtain good accuracy within a reasonable time.

[1] *Manuscript received September 5, 1931.*
Contribution from the Department of Physics, Dalhousie University, Halifax, N.S.

[2] *Demonstrator in Physics, Dalhousie University.*

Apparatus

The counting chamber consisted of a brass cylinder having a removable end plate for the introduction of the source. A hole in the opposite end was covered with a glass disk on which the zinc sulphide screens were waxed. The source was held in such a position that the screen was at its centre of curvature. The whole apparatus was connected to a manometer and pump, so that the pressure could be varied. Air was admitted through a drying tube. The temperature of the air enclosed was read by a thermometer sealed into the wall of the cylinder. A mechanical shutter could be interposed between the source and the screen.

The source was uranium oxide, U_3O_8. It was necessary to remove radium, ionium and polonium, all of which gave rise to α-particles. The rest of the radioactive elements either give rise to β-particles or have very short periods and are not re-formed once the parent radium is removed.

Ionium was removed by adding a small amount of its isotope, thorium, and precipitating with oxalic acid. Radium was next removed by adding barium chloride and precipitating with sulphuric acid. Polonium was eliminated by adding bismuth nitrate and precipitating with hydrogen sulphide. The uranium nitrate was then converted to U_3O_8 by ignition and ground as fine as possible in an agate mortar.

After several unsuccessful attempts to obtain a thin layer of U_3O_8 on the glass, a scheme was devised whereby the material was blown on by means of an air stream. The glass segment was previously coated with very soft wax. The air stream was allowed to pass over the material held in a glass tube, an electric tapper keeping the particles agitated. This scheme automatically sorted out the finest particles and deposited them in a thin layer on the surface of the glass. A source could be prepared very quickly and showed an even deposit under the microscope. The weight of uranium oxide deposited on the source was found to be 0.00045 gm. per cm^2 corresponding to 0.21 cm. of air approximately. The source had an area of 37.6 $cm.^2$ and was placed at a distance of 4.51 cm. from the screen.

The screens were prepared by smearing a cover glass with a very small quantity of castor oil, sifting a small amount of zinc sulphide (Glew's) on the oil and shaking off the excess. Several screens were tried. The one used in the final determination gave good scintillations at a distance of 3.15 cm. and at the same time had a very low natural effect of about one scintillation every 10 min.

The maximum number of α-particles to be expected within a circle of 0.29 cm. diameter, using the source described, was calculated. Then the area covered by the zinc sulphide was estimated by taking a micro-photograph of the screen, projecting it on squared paper and outlining a number of the crystals. When allowance was made for the area not covered with crystals, the efficiency of the zinc sulphide was 72%.

The screen was observed with a microscope (Watson "Holos" objective NA 0.45) giving a field 0.29 cm. in diameter. A weak light illuminated the

screen serving to keep the eye focussed. The natural effect of the screen was measured for each determination by closing the shutter. These "zero counts" were taken over approximately the same length of time as the direct counts. The mean temperature and the pressure were read and recorded with the aid of a dim lamp. The distance between the source and screen was calculated at 15° C. and 760 mm. in the usual way, all formulas being reduced by a set of nomograms, so that the pressure could be changed rapidly in the dark room.

Results

A series of counts was made at points ranging from 2.72 to 3.15 cm. equivalent distance from the source. This work was tedious, and required a total of about 60 hr. counting. The values were grouped, averaged and nine points obtained. The above procedure was essential to increase the accuracy of each point, since the probable error depends on the number of scintillations counted.

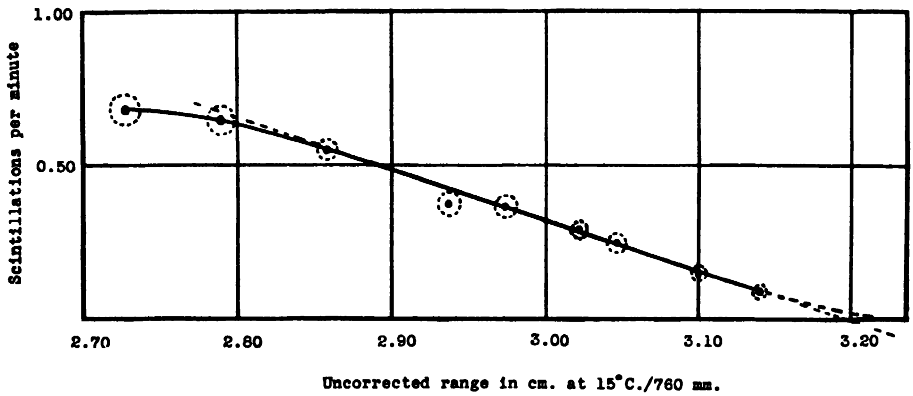


FIG. 1. *Uncorrected range of α-particles from uranium II.*

In the curve, Fig. 1, the ordinates represent the number of scintillations per minute, and the abscissas the distances from the source at 15° C. and 760 mm. The radius of each circle is proportional to the probable error.

The range was obtained in the usual way by drawing the tangent to the curve at the point of inflection, and producing it to intersect the abscissa. The point of intersection is seen to be at 3.20 cm. with an error of ±0.02 cm. due to various possible slopes of the tangent.

Correction for Absorption by the Source

The fraction of the total number of tracks which are longer than $R+x$ is

$$z = \frac{1}{h\sqrt{\pi}} \int_x^\infty e^{-\frac{x^2}{h^2}} dx,$$

where h is a parameter determining the straggling of the curve. From this equation Laurence (7) has shown that a length of

$$\frac{1}{2}h\sqrt{\pi}+\frac{1}{2}m-m\left[2\left(\frac{2}{\sqrt{\pi}}\int_{0}^{\frac{m}{2h}}e^{-y^2}dy\right)\right]^{-1}$$

must be added to the range to correct for absorption by a source of finite thickness, where m is the thickness of the active layer in terms of cm. of air.

The distance from the range to the point of inflection is $\frac{h\sqrt{\pi}}{2}$. From this h was calculated for the particular curve. Substituting this together with the value of m for the source used, gives 0.09 cm. to be added to the intercept.

The error in measuring the radius of curvature of the source, in drawing the tangent and in reading the manometer amounts to less than ± 0.08 cm. equivalent distance in the range, giving the final range of the α-particles to be 3.29 ± 0.08 cm. at 15° C. and 760 mm.

This confirms Laurence's value of 3.28 ± 0.03 cm. for the range. It will be noted that the accuracy in his determination is considerably greater than that obtainable in this experiment.

Remarks

Before making the final determination a previous trial experiment had been performed, using a screen less sensitive toward the end of the range, and having a large natural effect. The range obtained was considerably shorter than the final one. The errors in the first case were large, but not great enough to explain the difference.

Curie (2) has concluded that ranges found by scintillation methods fall short of those found by the Wilson chamber. Her conclusions are based on a comparison of the scintillation curve for Ra F obtained by Rothensteiner (10) and her own curve for Ra F based on Wilson chamber data. The work of Destriau (3) on the scintillation curve for Ra F lends further support to Curie's theory.

Recently, however, Philipp (9) has obtained the range of Th C^1 by scintillations in good agreement with Meitner and Freitag's (8) value found with a Wilson chamber, the errors in his determination being quite low. Bearing in mind the types of screen used in the trial and final experiments on uranium II, it would seem, as Rutherford (12, p. 113) has recently suggested, that the earlier attempts exaggerated the straggling. The curve obtained by the scintillation method approaches that obtained with a Wilson chamber as the screen becomes more sensitive and the natural effect decreases; provided the optical system is good, so that feeble scintillations are not overlooked.

The Geiger-Nuttall Relationship

By applying the Geiger-Nuttall relationship to Laurence's value of 3.28 cm. for the range of uranium II, the transformation constant was calculated to be 7.9×10^{-13} sec^{-1}, giving a period of 28,000 years.

Since the error in his determination of the range is probably no greater

than ±0.03 cm. it follows from the curve that the period is no greater than 43,000 years.

Recently the period of uranium II has been measured directly by Walling (14) and by Collie (1). Collie places a lower limit of 10^6 years for the period. Walling arrives at a value of 340,000 years with a calculated error of about 15%.

When Walling's value of 340,000 years and the range as given by Laurence are plotted, the resulting point shows a deviation of 4.0% in range from the Geiger-Nuttall curve, while the remainder of the uranium family show a mean deviation of only 0.8%. The difference here is greater than the experimental errors permit. This is shown in Fig. 2 where the circles indicate the errors in range. Uranium II is the only member of the family which is seriously out of line with the Geiger-Nuttall curve. For this reason it is desirable that further measurements of both range and period be made.

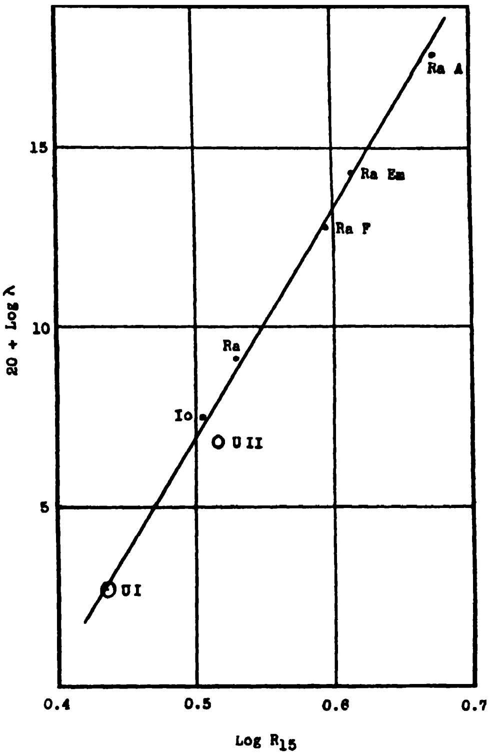


FIG. 2. *The Geiger-Nuttall relationship.*

Acknowledgment

The author wishes to thank Dr. G. H. Henderson for his encouragement and advice.

References

1. Collie, C. H. Proc. Roy. Soc. Lond. 131: 541-553. 1931.
2. Curie, I. Ann. phys. 3: 299-401. 1925.
3. Destriau, G. J. phys. radium, 2: 148-158. 1931.
4. Geiger, H. Z. Physik, 8: 45-57. 1922.
5. Geiger, H. and Nuttall, J. M. Phil. Mag. 23: 439-445. 1912.
6. Gudden, B. Z. Physik, 26: 110-116. 1924.
7. Laurence, G. C. Phil. Mag. 5: 1027-1038. 1928.
8. Meitner, L. and Freitag, K. Z. Physik, 37: 481-517. 1926.
9. Philipp, K. Z. Physik, 37: 518-528. 1926.
10. Rothensteiner, J. P. Ber. der Akad. Wiss. Wien, 125: 1237-1252. 1916.
11. Rutherford, E. Phil. Mag. 4: 580-605. 1927.
12. Rutherford, Sir E., Chadwick, J. and Ellis, C. D. Radiation from radioactive substances. Cambridge University Press, 1930.
13. Taylor, T. S. Phil. Mag. 26: 402-410. 1913.
14. Walling, E. Z. physik. Chem. Abt. B 10: 467-475. 1930.
15. Ziegert, H. Z. Physik, 46: 668-715. 1928.

RAMAN EFFECT OF BENZENE AND TOLUENE UNDER HIGH DISPERSION AND RESOLVING POWER[1]

BY LESLIE E. HOWLETT[2]

Abstract

This paper is a report of work carried out at the Macdonald Physical Laboratory on the Raman effect of benzene and toluene. The six-prism spectrograph, used by Dr. J. S. Foster for his work on the Stark effect, was employed in the investigation. It was found that a number of entirely new lines were observed in the Raman spectrum of both liquids. Some of these are due to the separation into components of previously observed lines; others are entirely new. Accurate measurements are given of the Raman frequency shifts and comparison with other results is afforded.

Introduction

Some previous work (1, 4, 5, 6) has been done on the examination of the Raman spectra of certain compounds under higher dispersion than is usually employed for this type of investigation. Hitherto the general result has been that the frequency differences characteristic of the molecules studied have been determined with greater accuracy but that no very new data have been revealed. It was felt by the writer that more work might be profitably done with the use of high dispersive and resolving power. The following discussion is the result of such investigations carried out on the much studied liquids benzene and toluene. The Raman spectra of these two substances have been very carefully studied, and the work has definitely shown that the use of spectrographs of high dispersive power is not only illuminating but quite necessary if the Raman spectra are to be thoroughly investigated.

Apparatus and Experimental Procedure

The spectrograph employed for this work was the six-prism one used by Dr. J. S. Foster of the Macdonald Physics Laboratory for his investigations on the Stark effect (2). Dr. Foster very kindly placed this instrument at the disposal of the author during the past summer. Since the details of its construction have been adequately described elsewhere (2), they are omitted in the present paper.

The success of Raman investigations depends to a large extent on the quality of the exciting source. The mercury arc serves as a very convenient and intense source of exciting radiation. It has the disadvantage, however, that after it has been in operation for some time the continuous spectrum increases very appreciably and as a result faint Raman lines are masked. Eventually even moderately strong ones are indistinguishable from the continuous background. Since it was desired to carry out this research under the most favorable conditions a new quartz burner was obtained. The lamp used was one of the Hanovia Research models. The wisdom of the preceding precaution was

[1] *Manuscript received October 5, 1931.*
Contribution from the Macdonald Physical Laboratory, McGill University, Montreal, Canada.

[2] *Junior Research Physicist, National Research Laboratories, Ottawa, and holder, at the time, of a fellowship under the National Research Council of Canada.*

demonstrated by the fact that after about 150 hr. of operation the continuous spectrum had increased appreciably. At the end of 500 hr. it was strong enough to mask the fainter Raman lines.

The Wood method of irradiation was used in all experiments. Great care and special precautions had to be taken to have the whole set-up in perfect alignment, otherwise the loss of scattered light was so great that it was impossible to obtain a photograph of the Raman spectrum. Stray light had to be excluded with equal care.

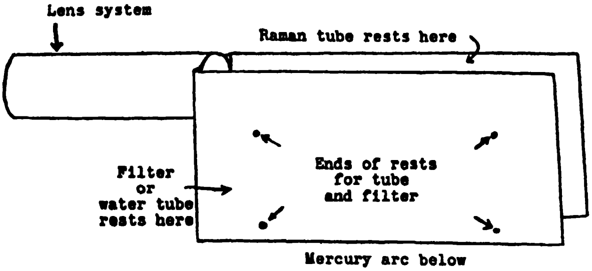


FIG. 1. *Diagrammatic sketch of apparatus.*

It may be of interest to describe briefly the method of setting up the apparatus used by the author. A rigid stand to hold the scattering liquid and the associated lens system was constructed. A sketch of this is shown in Fig. 1. It consisted essentially of two pieces of brass plate fixed together by four brass rods. To one end was attached a piece of telescope tubing to carry the lens system for condensing the scattered light on the slit of the spectrograph. An extra sleeve, which fitted over this, could be extended right up to the slit of the spectrograph. In this way all stray light was cut out from this part of the path taken by the scattered light. The inside of both tubes was painted black. The Raman tube itself was of Pyrex and fitted snugly between the two brass plates. It was supported by two of the brass rods used to fasten these plates together. The Pyrex window just fitted into the entrance of the telescope tubing. The window was stopped down somewhat by painting a small black ring around its outer edge. This was effective in cutting out reflected light. The top of the Raman tube and the inside surfaces of the brass plates were covered with silver foil. Immediately below the Raman tube another similar tube rested on the other two brass rods securing the plates together. This, when filled with water or a filter solution, served as a lens for condensing in the scattering liquid the light of the mercury arc which was just below this last tube. A blast of air was sent between the plates for cooling purposes. This proved very effective.

At the outset of the work this stand was set in perfect alignment with the collimator of the spectrograph by use of a small flashlight bulb set where the far end of the Raman tube would later come. Horizontal adjustment was then made to secure the proper relative positions for slit, lens and Raman tube. When this was done the stand was screwed rigidly in position. The line-up was checked at various times during the work but no further adjustments were required. The arrangement was found very convenient. It permitted change of the filter solution without disturbing the alignment or illumination in any way. If the arc went out during the exposure it could be started and put back in the same place with ease. In addition the iron arc could be photographed at the completion of the exposure by simply removing both tubes and

adjusting the position of the arc for focus on the slit. No change in the position of the lens took place. This last is an essential point to reduce as much as possible any chance of a shift in position of the iron lines on the plate with reference to the Raman spectra. Finally, it reduced the difficulty of alignment to a minimum. Great care was taken at the start and the work was finished. Thereafter only a check was required from time to time to make sure that all was still unchanged.

The plate-holder of the spectrograph is not curved and for this reason only a small section of the spectrum could be focussed on the plate at one time. On this account it was thought best to arrange for sharp focus of the region 4300-4700 Å. By so doing the higher frequency differences excited by 4046 Å and the lower ones excited by 4358 Å could be photographed on the same plate. The dispersion in this region ranged from 3-5.6 Å per mm. Ambiguity as to the origin of specific Raman lines was removed by the use of a quinine sulphate filter solution between the exciting source and the scattering medium during one of the exposures. Eastman-40 plates were found to be very suitable in the spectral region under consideration.

The iron arc was used as a comparison spectrum. It was superimposed on the Raman spectrum in the usual way. Measurements on the sharper and stronger Raman lines possess an accuracy of ± 0.3 cm^{-1}. In the case of the diffuse and faint lines the error should not exceed ± 1 cm^{-1}.

Merck's chemically pure benzene and toluene were used for the investigations. As a further precaution each liquid was freshly distilled before taking a Raman spectrogram.

Results and Discussion

Reproductions of the Raman spectrograms are shown in Fig. 2 and 3, respectively. The frequency differences are set forth in Tables I and II and are expressed in cm^{-1} in vacuum. Comparison is afforded between the results of the author and those of previous investigators. It will be noted that a fair number of extra frequencies are recorded by the writer which have not been previously reported. A number of these are, of course, due to the revelation of complexity under high dispersion of lines hitherto observed as simple. Others such as 807.2 and 842.4 cm^{-1} in toluene cannot be attributed to such an origin.

A glance at the reproduction of the Raman spectrograms and the tables of frequency shifts reveals how valuable is the use of high dispersion and resolving power. The principal advantages can be summed up under three headings:

1. Increase in the accuracy of the measurements.

2. The individual characteristics of the lines, *i.e.*, diffuseness, sharpness and gradation of intensity are more clearly shown.

3. A number of Raman lines are shown to be complex in structure, whereas smaller dispersion and resolving power had previously indicated them as simple. This may apply even to lines which are comparatively sharp under moderate and low dispersion.

Against these advantages is the objection to long exposures, which are

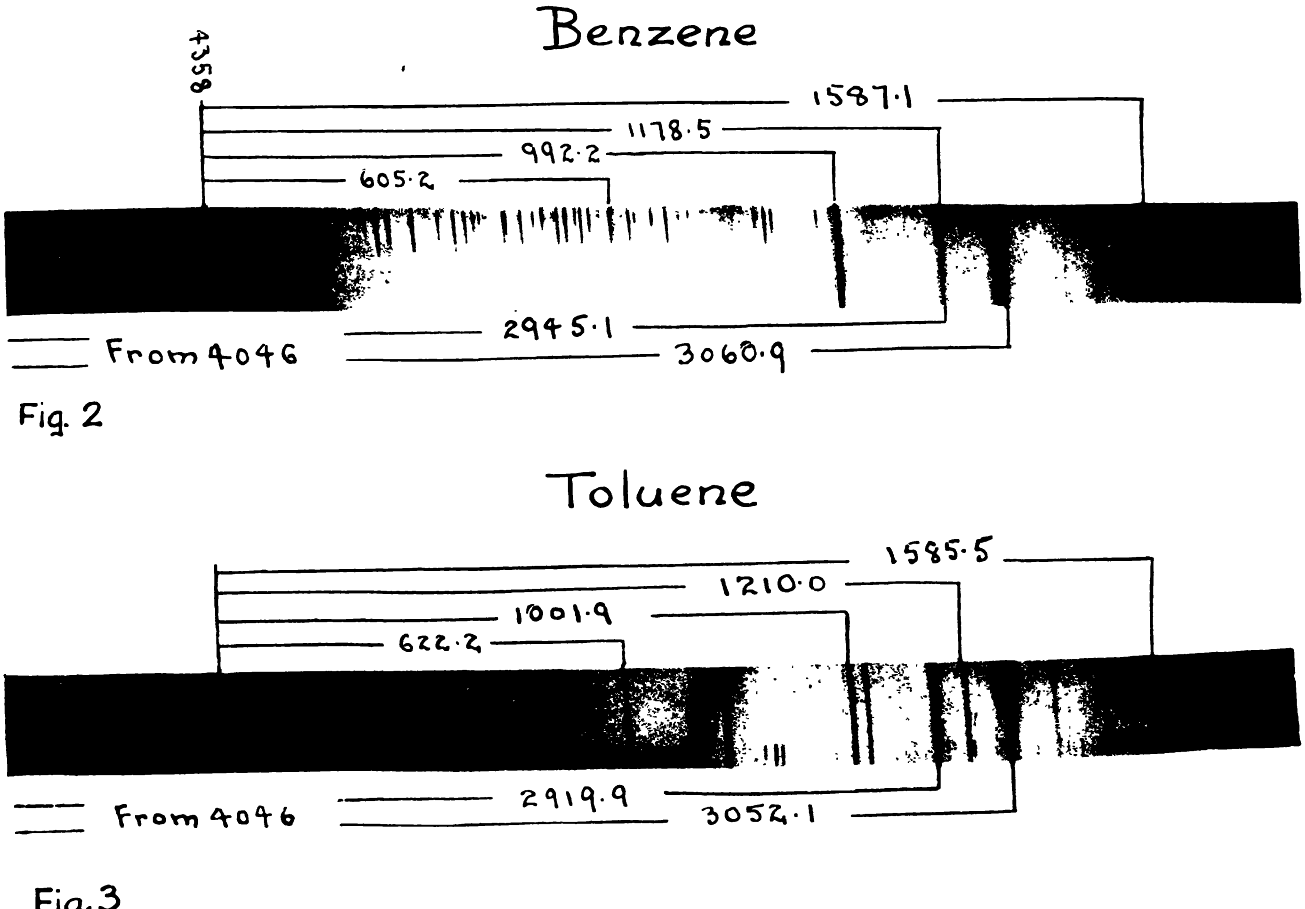


FIG. 2 AND 3. *Raman spectrograms of benzene and toluene.*

TABLE I

COMPARISON OF FREQUENCY DIFFERENCES FOR BENZENE OBTAINED BY VARIOUS INVESTIGATORS

Dabadghao (1)	Langer and Meggers (4)	Soderqvist (5)	Howlett	Dabadghao (1)	Langer and Meggers (4)	Soderqvist (5)	Howlett
3184.0 (2)	3185.0	3184.8 (2)	3186.1 (1)	—	—	—	1005.3 (0)
3160.5 (0)	—	3162.9 (1)	3163.1 (0)	—	—	—	998.8 (5)
3062.3 (4)	3060.1	3061.3 (4)	3060.9 (8)	991.2 (8)	992.1	991.3 (5)	992.2 (10)
3048.5 (1)	3045.4	3046.9 (1)	3047.1 (5)	—	—	—	983.9 (5)
2950.8 (2)	2946.9	2946.8 (2)	2945.1 (2)	—	—	—	980.3 (5)
1607.4 (1)	1604.5	1604.1 (1)	1604.9 (4)	848.7 (10)	848.6	849.1 (0)	849.4 (4)
1583.4 (1)	1585.2	1583.6 (1)	1587.1 (4)	603.9	605.7	604.6 (2)	605.2 (5)
1178.1 (1)	1177.3	1179.0 (1)	1178.5 (5)	—	—	—	—

NOTE:—*Exciting wave numbers: 24704.78, 22938.09 cm*$^{-1}$.
Numbers in parentheses are the visual estimates of intensities on a basis of 10 for the strongest Raman lines.

TABLE II

COMPARISON OF FREQUENCY DIFFERENCES FOR TOLUENE OBTAINED BY VARIOUS INVESTIGATORS

Langer and Meggers (4)	Soderqvist (5)	Howlett	Langer and Meggers (4)	Soderqvist (5)	Howlett	Langer and Meggers (4)	Soderqvist (5)	Howlett
—	—	3205.9 (0)	1604.5	1603.2 (1)	1604.7 (3)	—	—	968.3 (0)
3053.9	3053.7 (5)	3062.5 (4)	—	—	1585.5 (2)	—	—	842.4 (0)
—	—	3052.1 (5)	1379.4	1377.3 (1)	1379.5 (2)	—	—	807.2 (0)
—	—	3035.4 (3)	1208.7	1208.6 (3)	1210.0 (5)	785.8	785.6 (4)	786.7 (8)
—	—	3004.4 (0)	—	1154.1 (1)	1180.3 (3)	622.6	621.2 (0)	622.2 (3)
—	2981.2 (1)	2982.3 (0)	—	—	1156.5 (3)	521.0	519.3 (2)	519.8 (3)
2920.2	2919.6 (2)	2919.9 (4)	1031.3	1027.6 (2)	1027.7 (6)	—	332.6 (0)	335.0 (0)
—	—	2865.8 (0)	1003.3	1001.6 (5)	1001.9 (10)	217.3	217.5 (1)	216.7 (4)
—	—	1628.5 (00)	—	—	992.1 (3)	—	—	—

TABLE III

COMPARISON OF FREQUENCIES OF BENZENE AND TOLUENE

Benzene	Toluene	Benzene	Toluene	Benzene	Toluene	Benzene	Toluene
3186.1 (1)	3205.9 (0)		1628.5 (00)	1005.3 (0)	1027.7 (6)	604.6 (5)	622.2 (3)
3163.1 (0)	3062.5 (4)	1604.9 (4)	1604.7 (3)	998.8 (5)	1001.9 (10)		519.8 (5)
3060.9 (8)	3052.1 (5)	1587.1 (4)	1585.5 (2)	992.2 (10)	992.2 (3)		
3047.1 (5)	3035.4 (3)			983.9 (5)			335.0 (0)
	3004.4 (0)		1379.5 (2)	980.3 (5)	968.3 (0)		216.7 (4)
	2982.3 (0)		1210.0 (5)				
2945.1 (2)	2919.9 (4)	1178.5 (5)	1180.3 (3)	849.4 (4)	842.4 (0)		
	2865.8 (0)		1156.5 (3)		807.2 (0)		
					786.7 (8)		

necessitated by powerful spectrographs and the almost excessive care which is necessary in the alignment of the whole experimental arrangement to prevent undue loss of light. The first is not a really serious objection. If the spectrograph is set up on a good solid base in a place where temperature variations

are not wide, and a further precaution is taken to have a thermostatic device in the prism chamber, the greater part of the objection to long exposures is removed. If a mercury arc is used as the source of incident radiation one can operate this with very little attention for a long period of time. To set up the outfit for an exposure requires considerably more care and attention, but the improved results, it is felt, are well worth this added difficulty.

It is not in general possible to obtain anywhere near the precision of which one's instrument is capable when measuring Raman lines. The general diffuseness and faintness of many lines renders it impossible to approach the real precision of the instrument. It is felt, in this connection, that some have claimed greater accuracy for their results under low dispersion than was justified. The variation of the measurements on the same substances by different writers bears out this statement. Even with the large dispersion and resolving power employed in these experiments it was found that the average spread of the measurements made on the same line at different times but with equal care amounted on the average to about 1.1 cm^{-1}. In some cases it amounted to as much as a 2 cm^{-1}, in others not more than 0.6 cm^{-1}.

The high dispersion and resolving power shows with great clarity the variation in the individual characteristics of the lines, *i.e.*, sharpness, diffuseness, gradation, etc. Some lines have the sharpness of the incident exciting radiation, others have a width which runs to several Ångstroms or more. In the case of the latter, where there is no question of two Raman lines, coming from different exciting lines, being superimposed, there seems to be no departure from symmetry, *i.e.*, there seems to be no shading off either to the red or to the violet. This diffuseness is due, it is suggested, to an unresolved rotational structure superimposed on a pure vibration. The fact that some lines are quite sharp (these are in the minority) and others are diffuse or very diffuse seems to imply that partial rotations are possible. It is intended to study further the present plates for possible band systems.

In benzene all Raman lines except the group at 1,000 cm^{-1} are more or less diffuse. Those in the neighborhood of 3,000 cm^{-1} show the characteristic most markedly. The frequency 3047.1 is the most diffuse of these and its edges are poorly defined. The very strong frequency 3060.9, although broad has more sharply defined edges. The lines representing frequencies 1604.9 and 1587.1, characteristic of the double bonded carbon atom in the ring structure, are narrower than 3060.9 and yet can still be termed quite broad. Frequencies 604.6 and 849.4 are of about the same order of diffuseness; 1178.5 is somewhat sharper. The group near 1,000 cm^{-1} stands out unique in sharpness. These lines are of the same order of sharpness as the incident radiation. Two of them, 980.3 and 983.9, approach the sharpness of iron lines.

In toluene the lines representing the hydrogen frequencies are also broad and diffuse. The frequency 2865.8 is especially broad extending over 8-10 Å. The line 3052.1 is sharply defined, broad and quite intense. It resembles very strongly in appearance 3060.9 found in benzene. The other hydrogen lines are all diffuse but of varying degree. Of the remaining lines 216.7 is extremely broad and diffuse, while 786.7, 992.2, 1001.9, 1027.7 are comparable for

sharpness with the incident radiation; the others are moderately broad with 519.8 and 1210.0 standing out as the sharpest.

There is no indication of asymmetry in any of the Raman lines characteristic of benzene and toluene.

A glance at Table III in which the frequencies of benzene and toluene are arranged side by side shows how analogous are the two Raman spectra. The spectrum of toluene is the richer in Raman lines but the general grouping is the same. There is even a further similarity. A number of lines can be selected from each spectrum which correspond closely not only in frequency but in appearance. In Table III this point is illustrated by the underlining of such frequencies in each spectrum. It has been noted before that certain frequencies, very slightly modified, appear in the spectra of aromatic compounds. It appears even under high dispersion that, at least in the case of benzene and toluene, these lines have not only similar frequencies but have also the same appearance.

The groups of lines representing the hydrogen vibrations of both benzene and toluene have been shown in Fig. 4 and an approximate curve drawn to indicate the distribution of intensity. The similarity is rather striking as one would expect.

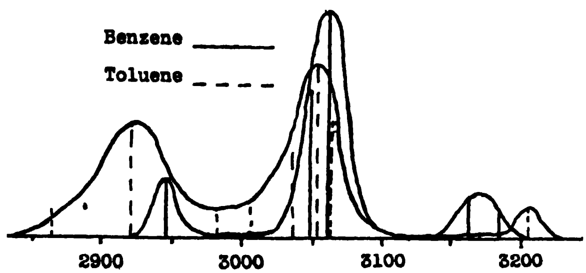


FIG. 4. *Visual estimates of the intensity distribution in the hydrogen vibrations of benzene and toluene.*

The group of lines near 1,000 is perhaps the most interesting. It is a strong line in this region which has hitherto been considered so characteristic of an aromatic compound. It is suggested that this frequency is closely linked with the symmetric vibration of the ring formation. It has always been noted by previous investigators as strong and sharp. The high dispersion and resolving power employed in these experiments brings out the fact that although less powerful instruments reveal it as sharp and simple it is really complex. In benzene it consists of five components, which are all quite sharp, of which some are comparable to the iron arc in sharpness. In toluene this group has three and possibly four components. There is some doubt as to whether 968.3 cm. should be included or not. Visually its appearance is quite unlike the others. It is quite broad. In proximity it is very close to the rest and for this reason should perhaps be included with them. Further investigations on other aromatic compounds would settle this point and provide interesting data on the modifications of this series as the hydrogen atoms of the benzene nucleus are substituted by various groups.

Such complexity, as is revealed in this case alone, indicates quite clearly, as must have been evident before, that postulated mechanical models of atoms bound by springs cannot possibly provide the basis for an accurate discussion of the Raman effect, however interesting and in many cases helpful these

models may be. As has been indicated by others and the present writer (3) these methods may be used with the aid of classical mechanics to predict the approximate order of Raman frequencies. The problem of dealing with Raman frequencies theoretically must be left to quantum mechanics for full and accurate solution. The complexity of the group at 1,000 cm^{-1} emphasizes this point. Such frequencies could not be deduced from the simple spring models but until the quantum mechanics is developed to the point where it can deal with such complicated molecules, the latter will, of course, serve a useful purpose. The gathering of careful and exact data on the Raman effect under high dispersive and resolving power will be important in that it will show of just what a complete theory has to take care. The fine points will not be revealed by low powered instruments although, of course, much important work has and will continue to be done by such instruments.

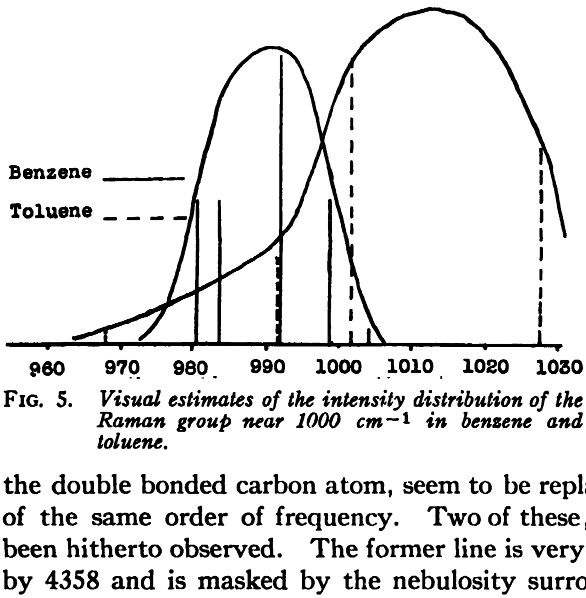


FIG. 5. *Visual estimates of the intensity distribution of the Raman group near 1000 cm^{-1} in benzene and toluene.*

Fig. 5 has been included to indicate diagrammatically the distribution and intensity of the lines of the group near 1,000 cm^{-1} in both benzene and toluene. It is shown that the intensity centre is shifted towards the higher frequencies in toluene.

The two lines of benzene representing 1604.9 and 1587.1 cm^{-1}, which originate in the vibrations of the double bonded carbon atom, seem to be replaced in toluene by three lines of the same order of frequency. Two of these, 1628.5 and 1585.5, have not been hitherto observed. The former line is very faint when due to excitation by 4358 and is masked by the nebulosity surrounding 4358, near which it appears, when excited by 4046. There seems, however, no question that it is a true frequency shift from 4358 as observed on the writer's plates.

The two lines 1180.3 and 1156.5 seem to take the place of the one 1154.1 observed by Söderqvist. It is interesting to note that Langer and Meggers did not observe any line at all in this region. On the present plates these two lines appeared quite as strong as the group near 1600 when a quinine sulphate filter was used. They are also quite strong when excited by 4046. This is not the first time that one author has observed fairly strong frequencies which another has failed to observe at all: acetone is another example. Such discrepancies are worthy of note. The writer suggests they may, to some measure, originate in the continuous spectrum which becomes so pronounced in the mercury arc after some use. He observed that on plates taken towards the end of the best period in the life of the mercury arc, the lines 1180.3 and 1156.5 and others in this region were becoming masked much more rapidly than those near 1600.

The justification of the assignment of the lines representing shifts 807.2, 842.4, 968.3, 992.1, 1585.5, 1628.5, 2865.8, 3004.4, 3035.4, 3062.5 and 3205.9 cm^{-1} rests on the use of a quinine sulphate solution as a filter between the arc and the scattering medium. The experimental data were not considered sufficient until at least two good spectrograms of each liquid had been obtained. The first one employed the full radiation of the mercury arc; the other used a quinine filter.

Acknowledgment

The author wishes to express his gratitude to Dr. A. S. Eve, Director of the Macdonald Physics Laboratory, McGill University, whose interest in this work made arrangements for its execution possible.

References

1. DABADGHAO, W. M. Indian J. Phys. 5: 207-217. 1930.
2. FOSTER, J. S. J. Franklin Inst. 209: 585-624. 1930.
3. HOWLETT, L. E. Can. J. Research, 4: 79-91. 1931.
4. LANGER, R. M. and MEGGERS, W. F. Bur. Standards J. Research, 4: 711-735. 1930.
5. SÖDERQVIST, J. Z. Physik, 59: 446-465. 1930.
6. WOOD, R. W. Phil. Mag. 6: 729-743. 1928.

STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XXXIX. STRUCTURE OF THE CELLULOSE SYNTHESIZED BY THE ACTION OF *ACETOBACTER XYLINUS* ON GLUCOSE[1]

BY HAROLD HIBBERT[2] AND JACOB BARSHA[3]

Abstract

A description is given of the properties of the cellulose obtained from glucose by the action of *Acetobacter xylinus*.

Acetylation of the product gives a yield of 98.8% of a triacetate identical with cellulose triacetate, and the cellulose regenerated from the acetate is identical with the starting material. The triacetate, when spun dry from solution in chloroform, gives a silk-like fibre which on de-acetylation yields a fibre showing the same X-ray diffraction pattern as natural cellulose. Acetolysis of the acetate yields cellobiose octacetate.

Treatment of the triacetate with methyl alcohol containing HCl gives a yield of 94.1% of α- and β-methylglucosides, while on direct hydrolysis of the cellulose with a solution of zinc chloride in hydrochloric acid, a practically quantitative yield (99.5%) of glucose is obtained.

Simultaneous de-acetylation and methylation of a partially saponified acetate soluble in acetone gave trimethyl cellulose (yield, 84.6%). The latter, on hydrolysis with methyl alcohol containing HCl, yielded 2:3:6-trimethyl methylglucoside (yield, 92.3%) which, in turn, was converted into crystalline 2:3:6-trimethyl glucose (yield, 83.5%). The last two compounds were found to be identical in every way with the same products prepared from ordinary cotton cellulose. It follows from this that the cellulose obtained by direct bacterial synthesis from glucose is identical with natural cellulose.

Introduction

The constitution of cellulose, in so far as the purely chemical side of the question is concerned, seems now to be definitely established (9, 10). Hydrolysis of cellulose results in a quantitative yield of glucose (13, 17) and it is interesting to note that in the present investigation, for the first time, it has been possible to demonstrate the reverse reaction, namely, the polymerization of glucose to cellulose, by the action of bacteria, the simplest form of plant life. However, the mechanism by which cellulose is formed in the plant is still unknown and it would seem that some information regarding this mechanism might be obtained by studying the products of the action of bacteria on simple carbohydrates and related products.

The selection of the bacterial polymerization product from glucose as the first substance to be investigated seemed to be a natural choice. This substance has been shown, as indicated in the experimental part, to be identical

[1] *Manuscript received October 14, 1931.*

Contribution from the Industrial and Cellulose Chemistry Laboratories, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada and the Canadian Pulp and Paper Association.

Communicated to the Cellulose Division of the American Chemical Society at the annual Fall Meeting on September 1, 1931, at Buffalo, N.Y.

Since completion of this work an article has appeared by E. Schmidt, M. Atterer and H. Schnegg (Cellulosechemie 12: 235-241. 1931) dealing with the chemical properties of a synthetic cellulose obtained from sucrose by bacterial action. The authors, however, have restricted their investigation to X-ray analysis, hydrolysis and acetolysis.

[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*

[3] *Holder of bursary under the National Research Council of Canada, 1930-31.*

with natural cellulose. A large number of other carbohydrates and related compounds such as fructose, sucrose, mannitol and glycerol, have been submitted to the action of *Acetobacter xylinus* and the chemical constitution of the resulting membrane is being actively investigated.

A. J. Brown, one of the earliest investigators in this field, during his studies on *Bacterium aceti* (2, 3, 4), or as he sometimes called it "vinegar plant", found that the organism formed extremely tough membranes when cultivated in suitable nutrient solutions containing carbohydrates. The membranes thus formed yielded a reducing sugar upon hydrolysis with sulphuric acid and were readily soluble in a solution of cuprammonium hydroxide. These properties, and the results of combustion analysis, led him to believe that the membrane was cellulose. In his report (3) he stated, "This production of cellulose by a simple cell plant, and its use as a cell connecting medium, seems of great interest in view of the important part which cellulose plays in a similar manner in the more highly organized forms of the vegetable kingdom; and it appeared that any information that could be gained, as to the materials from which cellulose is formed by the "vinegar plant" might perhaps assist in better understanding of the complex reactions which take place in higher plants".

Brown was able to obtain a similar type of membrane from fructose, mannitol and glucose. He found that the one prepared from fructose yielded a dextrorotatory sugar upon hydrolysis with sulphuric acid.

Emmerling (7) obtained membranes by using Bertrand's Sorbose bacterium (1), the latter identical with Brown's *Bacterium xylinum*. These products were only slightly soluble in cuprammonium hydroxide, and contained between 2 and 3% of nitrogen. On treatment of the pellicle with hydrochloric acid a crystalline product was obtained which resembled glucosamine hydrochloride, and which led him to conclude that the original membrane did not consist entirely of pure cellulose, but that a chitin-like substance was also present.

C. A. Browne (5) found that a cellulose-like fermentation of sugar cane juice was of fairly frequent occurrence in Louisiana, and he described the causal organism as being probably identical with Brown's *Bacterium xylinum*. He investigated the cellulose membrane from sugar cane juice but was unable to confirm Emmerling's results.

Eggert and Luft (6) studied membranes prepared by E. Schmidt, who apparently obtained them by the action of *Acetobacter xylinus* on sucrose, and from the dried preparations obtained X-ray diagrams similar to those from β-cellulose. Ipatjef (14) measured the rotation in a solution of cupramonium hydroxide of presumably similar material and obtained a value quite similar to that for cellulose.

In no instance has a detailed study of the synthetic formation of cellulose from glucose by bacterial action been made, nor has there been any thorough chemical study made of the polymerized product. The purpose of the present investigation was, first, to examine the method of formation of the cellulosic material by the action of *Acetobacter xylinus*, and, secondly, to carry out an exhaustive chemical investigation of the products obtained from various carbohydrates and related products by the action of this organism with a view

to throwing more light on the true nature of plant synthesis.

Discussion of Results

The "cellulose" whose constitution is the subject of this investigation was prepared by the action of *Acetobacter xylinus* on glucose according to the method outlined in a previous communication (21).

It was possible, by washing, to obtain the "cellulose" free from reducing sugars and from nitrogen compounds. This latter fact stands in contrast to the observation of Emmerling (7) who obtained membranes containing between 2 and 3% of nitrogen. He assumed the latter was due to the presence of a chitin-like material in the membrane, but it now seems more probable that the nitrogen found was part of the nutrient material originally employed.

The air-dried membranes obtained in the present research still contained between 5 and 7% of moisture and about 0.3% of ash which could not be removed by boiling with water. Estimation of carbon and hydrogen on a sample dried at 100° C./15 mm. for 10 hr. gave values establishing the empirical formula $(C_6H_{10}O_5)_x$.

Owing to the compactness of the dried membrane, it proved to be somewhat more inert than natural cellulose to chemical reagents. The acetylation reaction was found to be the best means for bringing the membrane into a form such as would facilitate the determination of its chemical constitution. Acetylation with a mixture of acetic acid and acetic anhydride, using sulphuryl chloride as catalyst, produced an almost quantitative yield of a triacetate having the same properties as the triacetate prepared from cotton cellulose. A similar acetylation was also carried out using concentrated sulphuric acid as catalyst.

De-acetylation of "cellulose" triacetate by saponification with 2*N* alcoholic sodium hydroxide yielded a regenerated "cellulose" which, because of its powdery nature, proved to be more amenable to the action of hydrolytic agents.

Partial de-acetylation of the triacetate produced an acetate containing about 40% acetyl (CH_3CO-) which was completely soluble in acetone and similar in this respect to the commercial acetates used for the manufacture of artificial silk.

Dry-spinning of a chloroform solution of the triacetate yielded a silk-like fibre which on complete de-acetylation by immersion in 2 *N* alcoholic sodium hydroxide solution yielded a fibre of regenerated cellulose. X-ray examination of the latter proved its identity with natural cellulose.

Hydrolysis of the triacetate with methyl alcohol containing 0.75% HCl gave a 94% yield of a mixture of α- and β-methylglucosides. Direct hydrolysis of the "cellulose" with a hydrochloric acid solution of zinc chloride showed an almost quantitative conversion of the "cellulose" to glucose, thus proving the former to be composed entirely of glucose building-units.

A simultaneous de-acetylation and methylation of the acetone-soluble acetate gave a yield of 84% of trimethyl cellulose, the latter on hydrolysis with HCl and methyl alcohol yielding the characteristic 2:3:6-trimethyl

methylglucoside (yield 92.3%). Further hydrolysis with 5% HCl gave crystalline 2:3:6 trimethyl glucose (yield 83.5%).

The above data, a synopsis of which is given in the accompanying table, prove conclusively the identity of the synthetic cellulose in question with normal cotton cellulose.

TABLE I

COMPARISON OF THE PROPERTIES OF COTTON CELLULOSE WITH SYNTHETIC CELLULOSE OBTAINED BY THE ACTION OF *Acetobacter xylinus* ON GLUCOSE

Cellulose triacetate (15)	α- and β-Methyl glucoside obtained from cellulose triacetate (15)	Trimethyl cellulose (11)	2:3:6-Trimethyl methylglucoside (16)	2:3:6: Trymethyl glucose (16)
Cotton cellulose				
$\alpha_D = -22.3°$ (chloroform) (c = 0.8092) Yield: 99.5%	$\alpha_D = +106\text{-}108°$ (equilibrium rotation in methyl alcohol—HCl) (c = 0.970) Yield: 95.5%	$\alpha_D^{16} = -10.0°$ (chloroform) (c = 1.04) Yield: 85-98% M.p. = 215-216°C. $OCH_3 = 45.6\%$	$\alpha_D^{20} = +66.0°$ (chloroform) (c = 1.34) Yield: 95% B.p. = 115-118° C./0.5 mm. n = 1.4590	$\alpha_D = +66.5°$ (methyl alcohol—HCl) (c = 1.35) Yield: 86% M.p. = 94-104°C.
Synthetic cellulose				
$\alpha_D^{22} = -21.9°$ (chloroform) (c = 1.0140) Yield: 98.8%	$\alpha_D^{22} = +107.5°$ (equilibrium rotation in methyl alcohol—HCl) (c = 0.9996) Yield: 94.1%	$\alpha_D^{22} = -9.2°$ (chloroform) (c = 2.226) Yield = 84.6% M.p. 231-232°C. $OCH_3 = 44.0\%$	$\alpha_D^{23} = +64.4°$ (chloroform) (c = 2.495) Yield: 92.3% B.p. = 111.5-115° C./0.35-0.40 mm. n = 1.4560 (25°C.)	$\alpha_D = +65.2°$ (methyl alcohol-HCl) (c = 1.14) Yield: 83.5% M.p. = 95-97° C.

The synthetic cellulose is soluble in cuprammonium hydroxide, acidified zinc chloride solution and yields the usual viscose solution on treatment with carbon bisulphide and alkali.

Experimental

In collaboration with H. L. A. Tarr (21), the "cellulose" was prepared from glucose by the action of *Acetobacter xylinus*. It is to be noted that in the preparation of the membranes, a nutrient medium was used which did not itself produce growth with the bacteria so that the membrane may be regarded as being produced entirely from the carbohydrate employed. Control experiments were carried out in connection with each batch of material.

The product was made up of an infinite number of closely compacted membranes. The whole was enormously swollen so that a membrane, which in the swollen condition weighed about 200 gm., yielded only 2 gm. of dry substance, indicating that it had taken up about 10,000% of its own weight of water.

Purification was effected by boiling with repeated changes of distilled water

for about one week, at the end of which time the wash waters no longer gave a reducing action when tested with Fehling's solution. The washing process was interrupted at intervals and the membranes were centrifuged in a basket centrifuge so as to expel most of the water. It was possible to obtain, by this method, a product which was not only free from reducing sugars but one which was also free from nitrogen as shown by the usual sodium fusion test on the dried material.

The "cellulose" so obtained from glucose exhibits most of the properties of natural cellulose. It is completely soluble in Schweitzer's reagent, in zinc chloride solution, and is insoluble in all ordinary organic solvents. The dried membrane looks very much like parchment paper and shows no fibrous structure when examined under the microscope. It has great tensile strength. The product showed even greater resistance than cellulose to the action of the various chemical reagents employed in the determination of its structure.

When dried in air, the "cellulose" retains between 5 and 6% of moisture but this can be removed on heating for several hours, at 110°C./15 mm. in the presence of phosphorus pentoxide. Analysis:— Found: C, 44.79; H, 6.23%: calcd. for $(C_6H_{10}O_5)_x$; C,44.42; H, 6.2%.

Preparation of the Triacetate

Method (*a*). *Using sulphuryl chloride as catalyst* (*15*). Five grams of "cellulose" (moisture, 6.55%) was left in contact for 44 hr. at 18-20°C. with 37.5 cc. of glacial acetic acid. Then 37.5 cc. of glacial acetic acid, through which a dry stream of chlorine gas had been bubbled for 45 sec., was added to the mixture. After standing for half an hour, 90 cc. of acetic anhydride was added and SO_2 gas bubbled through the mixture for 60 sec. It was then stirred rapidly for one hour at room temperature. The reaction flask, which was fitted with a mercury-seal stirrer and reflux condenser, was immersed in a water bath at 65° C. and kept at this temperature with rapid stirring for three hours. The mixture was allowed to stand overnight at room temperature. The viscous syrup was diluted with 200 cc. of chloroform and a large excess of water (4 litres) added. The chloroform was then evaporated whilst the mixture was being stirred continuously. During this process, the acetate separated in granules which were washed with water until free from acetic acid, and then dried at 60° C./10 mm. To remove traces of free acetic acid, the product was extracted with ether in a Soxhlet extractor. Yield, 8.20 gm. (98.8% of theory).

The acetate was completely soluble in chloroform and in acetylene tetrachloride, somewhat soluble in pyridine and only partially soluble in acetone. It showed in chloroform solution $[\alpha]^{22}_{D} = -21.9°$ (c = 1.0140).

The acetyl content of the acetate was determined by the method of Murray, Staud and Gray (18). The "cellulose" acetate was weighed out using a 0.5-gm. sample and transferred to a 250-cc. Erlenmeyer flask. To this was added 20 cc. of pyridine. The flask was loosely stoppered and warmed at 53° C. with occasional shaking until the acetate had completely dissolved. This required about 10 min. To the flask was now added 20 cc. of 0.5 *N* sodium hydroxide solution while shaking gently to disintegrate any precipitate

formed. The flask was then tightly stoppered and placed in a bath at 53° C. for 30 min. At the end of this time, the sides of the flask were washed with 25 cc. of distilled water, two drops of phenolphthalein were added and the excess of alkali titrated with standard sulphuric acid until the solution just became colorless. Analysis:—Found: $COCH_3$, 44.4: calcd. for $(C_{12}H_{16}O_8)_x$; $COCH_3$ = 44.8.%.

Method (b). Using concentrated sulphuric acid as catalyst. Dry "cellulose" (10 gm.) was added at room temperature to a mixture of 37.5 cc. of glacial acetic acid, 37.5 cc. of acetic anhydride and 1 cc. of concentrated sulphuric acid in a glass centrifuge jar. Heat developed at the start and the jar was cooled in a beaker of cold water. The reaction mixture was allowed to stand at room temperature for half an hour and then stirred rapidly for 4½ hr. The viscous, golden-yellow syrup thus obtained was diluted with 150 cc. of glacial acetic acid and then centrifuged (4,000 r.p.m.). The solution, which was poured off from a small amount of undissolved material, was not quite clear and was clarified, after further dilution with 150 cc. of acetic acid, in the following manner: A Büchner funnel, carrying a very fine filter paper, was covered with a fairly thick layer of fuller's earth which had been deposited from an acetic acid suspension. The solution was poured on this and on applying suction came through perfectly clear. As the rate of filtration decreased, the surface of the fuller's earth was scraped lightly with a spatula and the rate of filtration thereby much increased.

The acetate was precipitated by allowing the clear solution to drop into 600 cc. of distilled water with rapid stirring. The precipitated product separated in a swollen condition and was separated by centrifuging. The acetate was again suspended in water, centrifuged and the process repeated using alcohol instead of water. The acetate, which still contained a great deal of absorbed liquid, was dried in the vacuum oven (60° C./15 mm.) in the presence of solid NaOH. It was dissolved in 175 cc. of chloroform and this solution placed in a 2-litre three-necked flask fitted with a mercury-seal stirrer and a condenser for downward distillation. A litre of distilled water was then added, and the mixture heated and stirred rapidly, thus removing the chloroform. The acetate was obtained in the form of snow-white granules which were filtered off and washed with water until the filtrate gave no precipitate with barium chloride. After drying, the acetate was extracted with ether in a Soxhlet extractor for five hours to remove free acetic acid. Yield, 16.2 gm. (91.5% of theory). $\alpha_D^{22} = -21.3°$ in chloroform (c = 0.6930).

Analysis: The material was dried at 78° C./15 mm. over P_2O_5. Found: $COCH_3$, 44.6; calcd.: $COCH_3$, 44.8%.

Regeneration of "Cellulose" from the Acetate

The triacetate (2 gm.) was covered with 50 cc. of 2*N* alcoholic sodium hydroxide solution and allowed to stand for 24 hr. The mixture was filtered and washed thoroughly with distilled water. The regenerated "cellulose" was suspended in water and a few drops of dilute HCl added to neutralize any adsorbed alkali. The mixture was again filtered and washed thoroughly until

no precipitate was given with silver nitrate solution. The product had the same fibrous appearance as the acetate from which it had been prepared. It was snow-white in color, non-reducing to Fehling's solution and was somewhat more reactive towards reagents than the original dried membrane. Its physical properties were identical with those of the original "cellulose" pellicle. Yield, 1.16 gm. (81.9% of theory).

Preparation of acetone-soluble "cellulose" acetate (19)

The triacetate (5 gm.) was heated with 60 cc. of 95% acetic acid and 2 gm. of sodium acetate for about 36 hr. at 98° C. This treatment produced a partial saponification of the acetate rendering it soluble in acetone. The soluble acetate was then precipitated from the solution in the same way as in the case of the triacetate.

*Spinning of Triacetate and X-ray Examination**

A 15% solution of the triacetate in chloroform was spun "dry", using the apparatus developed in the Kaiser-Wilhelm Institut für Faserstoffchemie, Berlin-Dahlem (8), and a silk-like fibre obtained. Complete saponification of this fibre by immersion in 2 *N* sodium hydroxide solution for 24 hr., followed by thorough washing, yielded a regenerated "cellulose" fibre. The latter on X-ray examination was found to be identical with fibres of natural cellulose.

Hydrolysis of "cellulose" triacetate with methyl alcohol. Formation of α- and β-methyl glucosides (15)

The triacetate (5 gm.) was heated with 75 cc. of methyl alcohol containing 1% of HCl in a sealed bomb-tube at 125° C. for 50 hr. At the end of this period, only a trace of solid remained undissolved and the liquid had assumed a golden-yellow color. The slight amount of acid remaining after this treatment was neutralized with silver carbonate, and the filtrate was decolorized with charcoal. The clear liquor was evaporated to a syrup under diminished pressure in a tared flask. The solvents were completely removed by evaporation at 60° C./10 mm. in a current of dry air to constant weight. It was found that crystallization could be accelerated by dissolving the resulting syrup in a small amount of hot alcohol and allowing the solution to stand for a few hours. The alcohol was then evaporated at 60-70° C. under reduced pressure. The material in the flask was completely crystalline and almost white. Yield, 3.17 gm. (94.1% of theory).

The crystals of α- and β-methylglucosides melted at 105-130° C. In a solution of methyl alcohol containing 1% HCl, the mixed glucosides showed a rotation of $\alpha_{\mathrm{D}}^{22} = +95.2°$ (c = 0.9996). After heating in a sealed tube at 100° C. for three hours, a portion of this same solution increased in rotation to +107.5°.

Fractional crystallization of the mixed glucosides from absolute ethyl alcohol yielded the characteristic crystals of α-methylglucoside melting at 164.5° C. In water $[\alpha]_{\mathrm{D}}^{20} = +157.2$ (c = 1.01). The mother liquor from the recrystallization now contained a large excess of the β-methylglucoside. Evaporation and heating in a bomb-tube for five hours at 125° C. with methyl alcohol con-

*The X-ray data are to form the subject of a separate communication.

taining HCl resulted in a re-establishment of the equilibrium and a constant rotation of +107.3° was found. This last result indicates that no compounds other than α- and β-methylglucosides were present.

Hydrolysis of "Cellulose" with Zinc Chloride-hydrochloric Acid

The method employed was that used by Hibbert and Percival (13) in the hydrolysis of cellulose. The hydrolytic agent was a solution of anhydrous zinc chloride (1 part) in two parts of concentrated hydrochloric acid (d. 1.180) and was of $d_4^{22} = 1.454$. The material used was regenerated "cellulose" prepared in the manner described above. This was used in preference to the "cellulose" itself because the former dissolved more rapidly in the solvent employed. About 0.25 gm. of the material, dried at 60° C./15 mm., was accurately weighed into a dry, glass-stoppered bottle and 50 cc. of the solvent added by means of a pipette. The mixture was shaken vigorously for about 20 min. at the end of which time solution had occurred. To obviate a slight cloudiness which obscured the reading of the polarimeter, the solution was filtered through a tared Gooch crucible with minimum suction to avoid loss of hydrogen chloride. The Gooch crucible was washed with distilled water and alcohol, dried at 60°C./20 mm., weighed and the increase in weight subtracted from the original weight of "cellulose" employed.

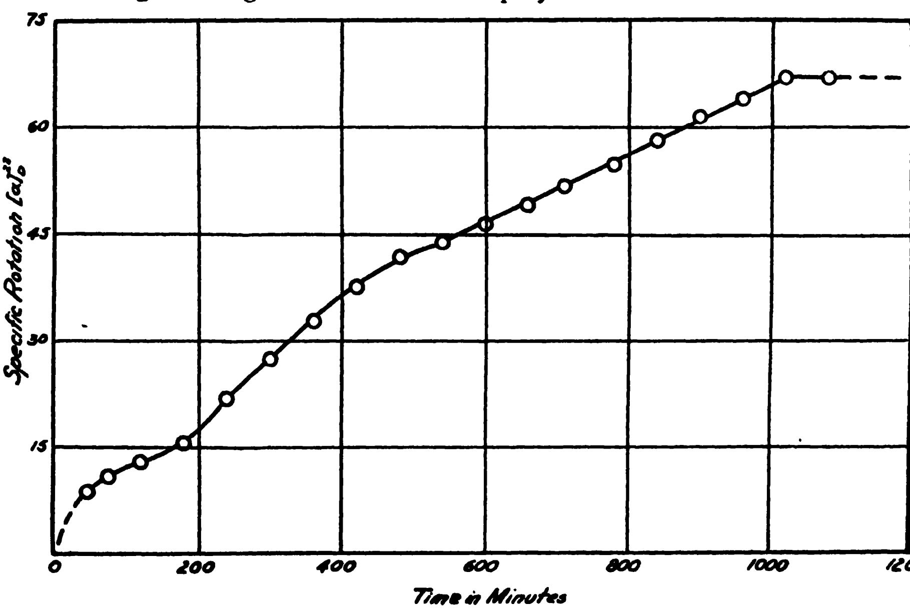


FIG. 1. *Hydrolysis of "cellulose" in zinc chloride-hydrochloride acid solution.*

The clear solution was transferred to a jacketed polarimeter tube kept at 23° C. ± 0.2° C., and readings were taken at intervals until the rotation became constant. $[\alpha]_D^{23}$ was calculated on the basis of glucose, *i.e.*, $[\alpha]_D^{23}$ observed ×162/180. The rotation of pure anhydrous glucose in the same solvent gave $[\alpha]_D^{23} = +67.3°$ (c = 1.0028). The results are given in Table II and plotted in Fig. 1.

TABLE II
EXPERIMENTAL RESULTS

Time, in min.	α_D^{23}	Time, in min.	α_D^{23}	Time, in min.	α_D^{23}	Time, in min.	α_D^{23}
0	0	300	27.3	660	49.2	1020	67.0
45	+ 8.9	360	32.8	720	51.9	1080	67.0
75	10.9	420	37.6	780	54.7	1140	+67.0
120	13.0	480	41.7	840	58.1		
180	15.7	540	43.7	900	61.5		
240	21.9	600	46.5	960	64.2		

NOTE:—*Concentration—0.4566 gm. of cellulose in 100 cc. of solvent.*

The above figures indicate quite clearly that the "cellulose" is hydrolyzed completely to glucose.

Preparation of Trimethyl "Cellulose"

Direct methylation of the dried membrane was attempted in a preliminary experiment. This was carried out according to the usual method of Haworth in which the membrane, cut up into small pieces, was treated with dimethyl sulphate and 30% NaOH solution. The reaction was carried out in a three-necked flask fitted with a stirrer and the reagents added from separate dropping funnels. At the end of the reaction, the starting material was unchanged in physical appearance. Analysis showed it to contain only 10.4% methoxyl. It appeared as if the same difficulty would be encountered here, as in the case of cotton cellulose, which as shown by Hess (12) requires ten methylations before the theoretical number of methyl groups can be introduced.

Recourse was then had to a simultaneous de-acetylation and methylation treatment of an acetone-soluble "cellulose" acetate according to the method which was applied recently by Haworth, Hirst and Thomas (11) to ordinary cellulose acetate. Dry acetone-soluble "cellulose" acetate (12.00 gm.; $CH_3CO = 39.4\%$) was dissolved in 240 cc. of acetone and treated with 385 cc. of 30% NaOH and 145 cc. of dimethyl sulphate in 10 equal portions at intervals of 15 min. The reaction was carried out at 56° C. in a one-litre three-necked flask fitted with a reflux condenser and a dropping funnel for the dimethyl sulphate. The NaOH solution was added from a dropping funnel through the condenser whilst the third neck was fitted with a mercury-seal stirrer. After the second addition, a gelatinous precipitate formed on the sides of the flask. This was returned to the reaction mixture by scraping with a glass rod and by vigorous stirring. The emulsion, which formed during subsequent additions, thickened towards the end of the reaction. Care was taken to maintain an alkaline reaction throughout the methylation. The acetone was then distilled off on the water bath, the mixture heated to 85° C. and filtered through a steam-jacketed Büchner funnel. The solid was washed with one litre of boiling distilled water. The light-brown granular product was dried, ground to a powder and triturated with 300 cc. of boiling water. After being heated for several hours on the steam bath, it was filtered again and washed with one

litre of boiling water. Yield, 8.44 gm. (92.2% of theory calc. for trimethyl cellulose). CH_3O, 40.7%; calcd. for $(C_9H_{16}O_5)_x$; CH_3O, 45.6%.

The product was then subjected to a second methylation using the same procedure as before. The reagents were added in 10 equal portions at intervals of 30 min. Yield, 8.20 gm. Analysis: Found: CH_3O, 42.2%.

This material was subjected to further methylation by means of Purdie's reagents. The reaction was carried out in a three-necked flask fitted with a spiral reflux condenser, the upper end of which was closed with a calcium chloride tube, a mercury-seal stirrer to keep the silver oxide in suspension, whilst the third neck, serving for the introduction of the pure, dry silver oxide, was closed by a cork.

The methylated product was dissolved in 100 gm. of methyl iodide in the flask, which was then immersed in a bath at 46-47° C. A clear solution was obtained. Silver oxide (40 gm., dried at 50° C./15 mm.) was added in four-gram portions every 30 min. At the beginning of the eighth addition, 25 gm. of methyl iodide was added to the reaction mixture which had become quite thick. After the last addition, the flask was heated for a further hour at 50° C. with vigorous stirring. Chloroform (approximately 100 cc.) was now added and the mixture stirred for 15 min. The silver salts were separated by centrifuging and then extracted four times with boiling chloroform (150 cc. each time) under reflux. The united chloroform extracts were dried over anhydrous magnesium sulphate, filtered and evaporated to a volume of 150 cc. The methylated product was obtained as a snow-white precipitate by dropping the chloroform solution, previously cooled to 0° C. into 700 cc. of cooled dry ligroin (b.p. 30-50° C.) while stirring rapidly at 0° C. It was filtered off, washed with more ligroin and dried over sulphuric acid in vacuum. It was dried further at 78° C./15 mm. over P_2O_5. Yield, 8.06 gm. Analysis: Found: CH_3O 43.6%.

A second methylation was carried out with Purdie's reagents exactly as described above. Yield, 7.75 gm. (84.6% of theory). The snow-white powder was soluble in chloroform and benzene; it dissolved slowly in ice-water but was insoluble in boiling water. Analysis:—Found: wt. 0.02185 gm. gave 0.07277 gm. AgI; CH_3O, 44.0; calcd. for $(C_9H_{16}O_5)_x$; CH_3O, 45.6%. In chloroform $[\alpha]_D^{22} = -9.2°$ (c = 2.226); in benzene $[\alpha]_D^{22} = -14.3°$ (c = 1.706). M.p., 231-232°C. For methylated cellulose with slightly lower methoxyl content than the theoretical, Haworth, Hirst and Thomas (11) also found a higher melting point and numerically smaller rotations than they found for completely methylated cellulose.

Hydrolysis of Trimethyl "Cellulose"; Preparation of 2:3:6-Trimethyl Methylglucoside (16)

Trimethyl "cellulose" (1.9 gm.) and 30 cc. of absolute methyl alcohol, containing 1% of HCl, were heated in a sealed tube at 100° C. for 95 hr. The solution was almost colorless and only a slight amount of flocculent material remained undissolved. The solution was neutralized with silver carbonate, filtered and decolorized with charcoal (Darco). The solution was evaporated

in a tared flask under diminished pressure and dried to constant weight at 100° C./15 mm. A colorless, viscous liquid remained in the flask. Yield, 2.03 gm. (92.3% of theory calculated for the conversion of trimethyl cellulose to trimethyl methylglucoside). The syrup was distilled at low pressure. A colorless, viscous syrup (1.50 gm.) was obtained. B.p. 111.5-115°C./0.35-0.40 mm. Refractive index, 1.4560 (at 25° C.). In chloroform $[\alpha]_D^{23} = +64.4°$ (c = 2.495). Analysis:—Found: wt. 0.02779 gm. gave 0.10933 gm. AgI; CH_3O, 52.0%; calcd. for $C_{10}H_{20}O_6$; CH_3O, 52.6%.

Preparation of 2:3:6-Trimethyl Glucose (12, 16).

Trimethyl methylglucoside (1.4 gm.) and 75 cc. of 5% hydrochloric acid were heated on the water bath under reflux for 11 hr. The solution was neutralized with barium carbonate, decolorized with charcoal and evaporated to dryness under reduced pressure. The residue was extracted with ether, dried with anhydrous magnesium sulphate and filtered. The ether solution was then concentrated to a volume of 25 cc. On cooling to −10° C., a portion of the 2:3:6-trimethyl glucose crystallized out and was filtered off. The mother liquor was evaporated under reduced pressure. The resulting syrup crystallized after seeding with a crystal from the first crop. Yield, 1.1 gm. (83.5% of theory); m.p. 95-97° C. The specific rotation in methyl alcohol became constant after catalysis with a trace of HCl. $[\alpha]_D^{24} = +65.2°$ (c. = 1.14). M. p. after recrystallization from dry ether, 105-108°C. Irvine and Hirst (16) found m.p., 104-108°C. Analysis:—Found: H, 8.36; C, 48.78%: calcd. for $C_9H_{18}O_6$; H, 8.2; C, 48.63%.

"Cellulose" Acetolysis

The procedure followed was that described by Spencer (20). One preliminary experiment was carried out, in which two grams of "cellulose" was treated with a mixture of 8 cc. of acetic anhydride and 0.2 cc. of concentrated sulphuric acid at 50° C. for 14 days. Treatment of the reaction product as outlined by Spencer yielded 0.75 gm. of crude cellobiose octacetate. After one recrystallization, a snow-white crystalline octacetate was obtained, m.p., 221-221.5° C. In chloroform $[\alpha]_D^{22} = +39.0°$ (c = 1.424).

Acknowledgment

The authors desire to express their best thanks to Dr. G. L. Clark for his kindness in carrying out the X-ray measurements on the regenerated cellulose. Their further thanks are due to Dr. H. L. A. Tarr for a liberal supply of the experimental material and to Mr. J. G. Morazain for his kind help in connection with the spinning of the acetate.

References

1. Bertrand, G. Compt. rend. 126: 842-844; 984-986. 1898.
2. Brown, A. J. J. Chem. Soc. 49: 172-187. 1886.
3. Brown, A. J. J. Chem. Soc. 49: 432-439. 1886.
4. Brown, A. J. J. Chem. Soc. 51: 643. 1887.
5. Browne, C. A. J. Am. Chem. Soc. 28: 453-469. 1906.
6. Eggert, J. and Luft, F. Z. physik. Chem. Abt. B. 7: 468-470. 1930.

7. EMMERLING, O. Ber. 32: 541-542. 1899.
8. FRANK, G. R., KRUEGER, H. E. and WOLFF, W. W. Zeit. Ang. Chem. 44: 342-348. 1931.
9. FREUDENBERG, K. J. Chem. Soc. Ind. 50: 287T-294T. 1931.
10. HAWORTH, W. N. Tenth Conference of the International Union of Chemistry, Liège. 1930.
11. HAWORTH, W. N., HIRST, E. L. and THOMAS, H. A. J. Chem. Soc. 824-829. 1931.
12. HESS, K. and WELTZIEN, W. Ann. 442: 46-60. 1925.
13. HIBBERT, H. and PERCIVAL, E. G. V. J. Am. Chem. Soc. 52: 3995-4005. 1930.
14. IPATJEV, V. N. Compt. rend. acad. sci. U.R.S.S. Ser. A, p. 381. 1930.
15. IRVINE, J. C. and HIRST, E. L. J. Chem. Soc. 121: 1585-1591. 1922.
16. IRVINE, J. C. and HIRST, E. L. J. Chem. Soc. 123: 518-532. 1923.
17. MONIER-WILLIAMS, G. W. J. Chem. Soc. 119: 803-805. 1921.
18. MURRAY, T. F., STAUD, C. J. and GRAY, H. LEB. J. Ind. & Eng. Chem. (Anal. Ed.) 3: 269-273. 1931.
19. OST, H. Z angew. Chem. 32: 66-70; 76-79; 82-89. 1919.
20. SPENCER, C. C. Cellulosechemie 10: 61-73. 1929.
21. TARR, H. L. A. and HIBBERT, H. Can. J. Research, 4: 372-388. 1931.

A SYNTHESIS OF THE METHYLTRYPTAMINES AND SOME DERIVATIVES[1]

BY RICHARD H. F. MANSKE[2]

Abstract

Because of the interest attached to N-methyltryptamine on account of its occurrence as an integral part of the calycanthine molecule, the free base and some of its derivatives have been synthesized. An account of the synthesis of N,N-dimethyltryptamine together with some carbolines derived from 1-methyltryptamine is also included. Finally, a detailed procedure for an improved preparation of tryptamine is given.

N-methyltryptamine has become of some interest because of its occurrence as an integral part of the alkaloid calycanthine. In a recent communication the author (5) recorded the degradation of calycanthine to benzoyl-N-methyltryptamine, but the free base was not obtained at that time and its preparation, together with some of its derivatives, is now placed on record.

Considerably more difficulty was encountered in the synthesis of N,N-dimethyltryptamine but sufficient was ultimately obtained for characterization. When tryptamine is treated with methyl iodide, all four possible products are obtained. The removal of the quaternary iodide is comparatively simple because of its sparing solubility in water or alcohol and the greater portion of the unchanged tryptamine can be separated from the mixture of bases on account of its sparing solubility in ether. Benzoylation of the residual mixture left the tertiary base intact and it was extracted from the crude product by means of dilute acid. The accumulated impurities were then removed by conversion to the picrate and the latter was recrystallized until pure. The picrate thus obtained consists of pale yellow needles sparingly soluble in water and melting at 168°C*. In melting point and composition this picrate closely resembles that of a base ($C_{12}H_{16}N_2$) isolated from *Withania somnifera* by Power and Salway (6, p. 496) but the question of identity was settled in the negative by the preparation of the free base which has the unexpectedly low melting point of 47°C. Furthermore, the hydrochloride could not be obtained crystalline although the pure base was used in its preparation. Aside from analysis the constitution of the base was proved by conversion to the previously described (5) quaternary iodide.

Another route which also led to the tertiary amine was via the quarternary chloride (from the iodide) by distillation *in vacuo*. The yield in this case as well as in the previous case was rather unsatisfactory and purification through the picrate was essential.

The physical properties of this series of bases are of some interest since the cases where all three representatives (primary, secondary and tertiary) have been obtained crystalline are extremely rare. Aside from a general effect in

**All melting points are corrected.*

[1] *Manuscript received October 29, 1931.*
Contribution from the National Research Laboratories, Ottawa, Canada.

[2] *Associate Research Chemist, National Research Laboratories.*

raising the melting point the indol nucleus is sufficiently removed from the nitrogen atom so that the effect of the successive introduction of methyl groups in lowering the melting point is probably not ascribable to any other cause. The melting points are: primary 117°, secondary 90°, tertiary 47°C. Parallel with the decrease in melting point a marked increase in solubility in organic solvents is observed. While tryptamine is soluble only with difficulty in boiling ether, the tertiary base dissolved rapidly in this solvent at 0°C.

In one attempt to methylate tryptamine with methyl iodide in acetone there was obtained, in addition to the quarternary iodide, a base or mixture of bases which yielded on benzoylation a beautifully crystalline product giving analytical figures in good agreement with $C_{20}H_{20}ON_2$. The substance gives a greenish blue color with Ehrlich's reagent, a fact which is indicative of substituents at positions 2 and 3 in the indol nucleus and probably also of ring closure. These properties and its mode of formation point to the substance being 2:2-dimethyl-3-benzoyl-2:3:4:5-tetrahydro-3-carboline, the gem-dimethyl group and the 2-carbon atom obviously originating in the acetone used as solvent. The mechanism of the synthesis recalls the usual isoquinoline synthesis, its facile occurrence being due to the great reactivity (3) of the 2-position in the indol nucleus.

Späth and Lederer (7) have described the synthesis of a number of carbolines from 1-methyltryptamine. The benzoyl derivative recently described by the author (5) has now been converted into the corresponding dihydro-carboline. Oxidation with chromic acid yields the carboline which exhibits the usual bluish fluorescence to a marked degree. Incidentally it may be pointed out that the phthalimido derivative of 1-methyltryptamine is admirably suited to the purification of the crude base owing to its ease of formation and sparing solubility even in boiling alcohol.

In view of the comparatively large amounts of tryptamine required for this investigation considerable attention was devoted to effecting improvements in yields at various stages, the final result being that the utility of the Ewin's (2) synthesis has been greatly enhanced.

The final condensation in the synthesis still leaves something to be desired in the matter of yields, but it must be kept in mind that in reality three reactions,—hydrolysis of the acetal, formation of the phenylhydrazone, and ring closure of the latter,—are carried out in one operation. The most serious obstacle heretofore has been the preparation of diethyl γ-aminobutyracetal from the difficultly accessible β-cyanoacetal (8). It has now been found that the comparatively simple preparation of the β-bromoacetal from acrolein has obviated much experimental difficulty in preparing the cyanide. The conversion of the bromide into the cyanide proceeds smoothly in boiling methanol, the troublesome autoclave conditions and the use of glycerol as solvent, essential in the case of chloroacetal, being entirely avoided. Furthermore, the yields are much better, and if the details given in the experimental section are observed the aminoacetal may be obtained in 85% yield from the cyanide.

The preparation of tryptamine is again detailed and finally attention may be

directed to an error in the recorded melting point (given as 138°C.) of benzoyltryptamine. The correct value (174°C.) is that first recorded by Asahina and Mayeda (1) and the incorrect value (due to Ewins) was erroneously introduced in writing the manuscript. It may also be pointed out that Ewin's melting point for tryptamine (145-146°C.) could never be confirmed. If there are in reality two crystal forms the higher melting form must of necessity be the most stable. Nevertheless, the distilled base which crystallized while still hot, that recrystallized from chloroform, that from alcohol, and the resolidified melt, all melted at 118°C. Asahina records 120°C., while Majima and Hoshino (4 p. 2045) give 114.5-115.5°C. On the other hand the melting points of the picrate (242-243° C.) and the hydrochloride (246°C.) given by Ewins are substantially within experimental error.

Experimental

N-Methyltryptamine

A rapidly cooled solution of 32 gm. of tryptamine in 300 cc. of chloroform was treated with an equal weight of methyl iodide and the mixture kept in cold water. When the first appreciable evolution of heat had subsided and a considerable amount of colorless syrupy salt had separated, the mixture was placed in an ice chest and allowed to remain for 3 days, during which time the salt largely crystallized. The solvent was decanted off and the residue dissolved in 120 cc. of ethyl alcohol. On cooling, a copious yield of the quaternary iodide was obtained. This was recrystallized once and it then melted at 197°C.

The combined mother liquor was freed of alcohol, dissolved in water, basified and the mixture extracted with chloroform. A further small amount of the quaternary iodide was obtained at this stage. It was recrystallized from alcohol (m p. 197°C.) and together with the first amount weighed 12.1 gm. The chloroform extract was clarified with sodium sulphate, the solvent distilled, and the residue distilled *in vacuo*. While still warm the distillate was treated with a small volume of chloroform and as crystallization proceeded ether was added. The mixture was then thoroughly cooled and the tryptamine filtered off and washed with ether; recovery, 15.1 gm.

It is possible to isolate N-methyltryptamine from the mother liquor of the tryptamine by fractional precipitation with petroleum ether, but the procedure is so tedious and the yields so low that a description is felt to be of no value. The mixture of primary, secondary, and tertiary bases which weighed 9.3 gm. in the above experiment was benzoylated as already described and the chloroform solution extracted with dilute hydrochloric acid (*A*).

Recovery and purification of the benzoyl derivative and boiling under reflux for 24 hr. with an excess of alcoholic potassium hydroxide (methyl alcoholic potassium hydroxide is inadequate as a hydrolytic agent, presumably because of the lower temperature of boiling) yielded the crude base. It was isolated from the alcohol-free hydrolysate by extraction with ether and removal therefrom with dilute hydrochloric acid. Regeneration of the base, extraction and distillation *in vacuo* yielded a colorless viscous distillate which was dissolved

in a little chloroform and cautiously treated with petroleum ether. Crystallization was rapid. After thorough chilling the base was filtered off, washed first with a little chloroform-petroleum ether and then with the latter solvent. N-methyltryptamine as thus obtained consists of stellate aggregates of needles, several isolated crystals showing rectangular form; m.p., 90°C.; yield, 4 gm. Analysis: Calcd. for $C_{11}H_{14}N_2$; C, 75.86; H, 8.05; N, 16.09%. Found: C, 76.07; H, 8.17; N, 15.90%.

The hydrochloride was obtained in colorless elongated plates with pyramidal terminations from alcohol-acetone or alcohol-ether; m.p., 180°C. Analysis: Calcd. for $C_{11}H_{15}N_2Cl$; Cl, 16.90%. Found: Cl, 16.96%.

It may finally be observed that hydrolysis of benzoyl N-methyltryptamine obtained from calycanthine yielded the base and hydrochloride identical with the above.

The picrate was obtained when an alcoholic solution of the base was treated with picric acid in the same solvent. Benzene was added and the solution evaporated to a small volume and the concentrate treated with much ether. The picrate then crystallized out in large plates closely resembling azobenzene in color. After thorough washing with ether it melted at 191°C. It is very sparingly soluble in hot water.

The phenylcarbamyl derivative of N-methyltryptamine was readily obtained by heating the base in chloroform with a slight excess of phenylisocyanate and evaporating to a small volume. Addition of ether caused the derivative in question to crystallize in large elongated hexagonal plates, which when recrystallized from methanol-ether melt sharply at 153°C. With Ehrlich's reagent it gives an immediate red color with a slight orange cast. Analysis: Calcd. for $C_{18}H_{19}ON_3$; N, 14.33%. Found: N, 14.47%.

N, N-Dimethyltryptamine Picrate

The acid extract (*A*) from the benzoylation of the tryptamines was basified and extracted with ether. Removal of the solvent and distillation of the residue yielded a pale yellow viscous oil which failed to crystallize under a variety of conditions; yield, 1.0 gm. It was therefore converted into the picrate which was twice recrystallized from alcohol, washed with ether, and then recrystallized from hot water in which it is sparingly soluble; m.p., 168°C. Analysis: Calcd. for $C_{18}H_{19}O_7N_5$; C, 51.80; H, 4.56; N, 16.78%. Found: C, 51.95; H, 4.75; N, 16.62, 16.53%.

N,N-Dimethyltryptamine

A hot aqueous solution of trimethyl-β-3-indolyl-ethylammonium iodide was treated with a 50% excess of freshly precipitated silver chloride and the mixture gently boiled for 15 min., during which time the larger lumps were frequently disintegrated with a stout glass rod. The silver halide was filtered off and the clear filtrate rapidly evaporated, preferably in a stream of air. The addition of a small amount of methanol and the cautious treatment of the solution with acetone yielded a copious crop of crystals of the quaternary chloride. The yield of crystalline product is well in excess of 80% if the procedure is rapidly carried out. Recrystallization by solution in a small

volume of methanol and cautious addition of acetone yielded colorless many-sided stout crystals melting at 193°C. The chloride in contrast to the iodide is extremely soluble in water and alcohol, but only sparingly so in acetone. Analysis: Calcd. for $C_{13}H_{19}N_2Cl$; Cl, 14.87%. Found: Cl, 14.06%.

Methyl chloride was removed from the quaternary chloride by slowly heating 5 gm. in a distillation flask in a vacuum kept below 1 mm. Excessive heating caused much frothing so the operation was conducted very slowly. There was considerable deep-seated decomposition and an appreciable amount of non-volatile resin remained in the flask. The crude distillate was dissolved in a small volume of methanol and an excess of dilute aqueous nitric acid was added. After removal of the methanol the turbid mixture was filtered through a wet filter and the acid filtrate extracted several times with choloroform to remove non-basic material. The tertiary base was then liberated by the addition of an excess of sodium hydroxide and the base extracted with chloroform. The dried extract was freed of chloroform by repeated evaporation with ethyl alcohol and then poured into a hot dilute solution of picric acid. A small amount of insoluble resin was filtered off and the filtrate slowly cooled. The picrate was thus obtained in pale yellow slender needles melting at 167°C., which after one recrystallization melted alone or admixed with a specimen obtained from the methylation of tryptamine at 168°C.

The purified picrate from 5 gm. of the quaternary chloride was suspended in a small volume of boiling water and treated with an excess of sodium hydroxide. The cooled mixture was extracted with ether and the extract thoroughly washed with aqueous sodium hydroxide and dried over potassium carbonate. Removal of the ether yielded a pale yellow oil (1.6 gm.) which solidified completely in a short time. After pressing out on tile and washing with a mixture of ether and petroleum ether it melted sharply at 47°C. The free base was obtained only in very fine ill-defined needles. It is extremely soluble in all organic solvents with the exception of petroleum ether.

A small amount treated with an excess of methyl iodide in methanol yielded the characteristic micaceous plates of the quaternary iodide, which after one recrystallization from methanol melted at 197°C., alone or admixed with an authentic specimen.

The hydrochloride could be obtained only as a pale yellow resin, which when dried in a vacuum desiccator over potassium hydroxide became porous and brittle. Analysis: Calcd. for $C_{12}H_{17}N_2Cl$; Cl, 15.79%. Found: Cl, 15.44%.

2:2-Dimethyl-3-benzoyl-2:3:4:5-tetrahydro-3-carboline

A solution of 4 gm. of tryptamine in 40 cc. of dry acetone was treated with an excess of methyl iodide. Some heat was evolved but no precipitation occurred. After several hours the acetone was removed on the steam bath, water was added and the slightly turbid solution filtered through a layer of charcoal. The colorless filtrate was basified with excess KOH and the precipitated bases removed by extraction with a mixture of ether and chloroform. A small amount of insoluble crystalline material was obtained which proved to be the quaternary iodide, m.p., 197°C.

The ether-chloroform extract was dried over sodium sulphate, evaporated somewhat and heated for four hours under reflux with an excess of benzoyl chloride and potassium carbonate. The chloroform solution was thoroughly washed with water and with dilute hydrochloric acid and dried over potassium carbonate. On evaporation and treatment with a little acetone and ether, the chloroform solution yielded about 0.7 gm. of very stout brilliant octahedra which after recrystallization from acetone, in which the substance is sparingly soluble, melted sharply at 285°C. Analysis: Calcd. for $C_{20}H_{20}ON_2$; C, 78.94; H, 6.58; N, 9.21%. Found: C, 78.77; H, 6.57; N, 9.25%.

3-(β-Phthalimidoethyl)-1-methylindol

A mixture of 7 gm. of crude 1-methyltryptamine and 12.5 gm. of phthalic acid was slowly heated to 230°C. in an oil bath. The somewhat cooled mixture was treated with 100 cc. of boiling alcohol and the solid broken up with a glass rod. The mixture was then heated under reflux for an hour, cooled, filtered and the solid washed with cold alcohol; yield, 10 gm. A small amount was recrystallized by solution in a large volume of hot acetone and rapidly evaporating the filtrate. On cooling, a solid mass of minute needles was obtained which after filtering, washing with alcohol, and drying melted sharply at 177.5°C. The substance is practically insoluble in cold alcohol and only sparingly in hot acetone. Analysis: Calcd. for $C_{19}H_{16}O_2N_2$; C, 75.00; H, 5.26; N, 9.21%. Found: C, 74.92; H, 5.36; N, 9.35%.

The phthalimido derivative when treated with hydrazine hydrate in the usual manner yielded 1-methyltryptamine quantitatively; b.p., 154°C./1 mm. All attempts to obtain the latter in a crystalline condition failed. Exposure in a sealed tube to the native climatic conditions for six months during which time the temperature frequently fell to -30°C. failed to cause crystallization.

The hydrochloride is readily obtained in colorless needles by neutralizing a solution of the base in alcohol with hydrochloric acid and cautiously adding acetone. Recrystallized from methanol-acetone the salt melts sharply at 198°C.

1-Methyl-2-phenyl-4:5-dihydro-3-carboline

A solution of 2.78 gm. of benzoyl-1-methyltryptamine in 35 cc. of chloroform was heated under reflux with 10 gm. of phosphorus oxychloride for one hour. The solvent and excess oxychloride were largely removed by evaporation in a current of air and the residue decomposed with ice. Some residual chloroform was then boiled off, and the turbid solution filtered through a layer of charcoal. The intensely yellow filtrate was basified and the base extracted with ether, the extract was dried over sodium sulphate, evaporated to a small volume and crystallization induced by cautious addition of petroleum ether. The microscopic crystalline base was filtered off and washed with ether-petroleum ether (1:1) and finally with petroleum ether; yield, 2.3 gm. The substance is colorless and melts sharply at 94°C. Alcohol readily dissolves it to a yellow solution with a pale greenish fluorescence. Analysis: Calcd. for $C_{18}H_{16}N_2$; C, 83.08, H. 6.15, N. 10.77%. Found: C, 83.02; H, 6.14; N, 10.88%.

The hydrochloride readily crystallizes from alcohol-ether or alcohol-acetone

in golden yellow elongated plates, melting at 237°C. to an orange-colored liquid which resolidifies on cooling.

1-Methyl-2-phenyl-3-carboline

A small amount of the dihydro-base was dissolved in dilute sulphuric acid and treated while hot with a solution of chromic acid. A very sparingly soluble chromate was precipitated which gradually dissolved as oxidation proceeded. The filtered solution was basified and the mixture of chromium hydroxide and base filtered off, washed with water, dried and extracted with hot ethyl acetate. The base obtained from the extract did not crystallize. It was converted into the hydrochloride and the latter recrystallized from acetone by adding ether to the concentrated solution. The hydrochloride melts at 278°C. and is very soluble in water or alcohol, yielding a solution with an intense bluish fluorescence. Analysis: Calcd. for $C_{18}H_{15}N_2Cl$; C, 73.34; H, 4.75; Cl, 12.05%. Found: C, 73.02; H, 5.12; Cl, 12.05%.

The picrate is insoluble in water and was recrystallized from hot alcohol in which it is sparingly soluble; m.p., 234°C.

Diethyl β-Cyanopropionacetal

Six moles (275 gm.) of absolute alcohol contained in a flask provided with an inlet tube, a dropping funnel, and a calcium chloride tube, was treated with a stream of dry gaseous hydrogen bromide until 2.75 moles (220 gm.) had been absorbed. The mixture had to be kept cool during this addition since an elevation of temperature converts a portion of the alcohol into the bromide. The mixture was then cooled in salt and ice and 2.5 moles (140 gm.) of acrolein was slowly added, care being taken to prevent local superheating. The mixture was allowed to remain in ice overnight, neutralized with an excess of precipitated calcium carbonate, and about 300 cc. of dry ether was added. The mixture was again allowed to remain in ice overnight and the ethereal layer then decanted from the pasty calcium bromide solution. A small amount of calcium carbonate was added to the ether solution and the solvent then removed *in vacuo*. To the residue, dissolved in 300 cc. of methanol, there was then added 3 gm. of sodium iodide and 2.75 moles (138 gm.) of sodium cyanide dissolved in the minimum volume of water. The mixture was boiled under reflux for 15 to 16 hr., the solvent distilled off on a steam bath and the residue extracted with about 600 cc. of ether in several portions. The extract was freed of ether and the residue fractionally distilled *in vacuo*, through a good glass column. Two main fractions were obtained: (I) up to 97°C./15 mm. (II) 97 to 108°C./15 mm. (mostly at 104-106°C.). Each fraction was redistilled and the higher portion of the first added to the second. The second fraction consisted of the desired nitrile and was conveniently collected over a 2° range. Some boiling points (not corrected) with the corresponding pressures were: 84-85°C./7 mm.; 97°C./11 mm.; 105°C./15 mm. The lower fraction contained a considerable amount of unchanged bromide which may conveniently be added to a subsequent preparation. The yield of nitrile varied from 40 to 60%, depending upon the amount of unchanged bromide recovered.

Diethyl γ-Aminobutyracetal

A solution of 79 gm. of β-cyanopropionacetal (0.5 mole) in 1000 cc. of absolute alcohol was treated with 70 gm. of sodium (3 moles) in large pieces, the mixture being cooled in running water. When the first vigor of the reaction had subsided, the flask was heated on a steam bath until all the sodium had dissolved. The somewhat cooled mixture was then treated with 200 cc. of water and as much alcohol as would distil was removed on a steam bath through a short column. The first distillate was discarded. The residue was distilled under reduced pressure until no more distillate could be obtained, and the distillate fractionated through a good column from a steam bath. The residue was then distilled through a long column *in vacuo*. With considerable care this distillation may be so carried out that a very sharp separation of the water is possible. The yield of a product of 3° boiling range was 67 to 68 gm. (85% of theory).

When methanol was substituded for ethyl alcohol the mean yield of four runs was 71%. The proportions were: nitrile, 157 gm.; methanol, 1500 cc.; sodium, 140 gm. The pure substance boils at 84°C./11 mm. or at 93°C./15 mm.

Tryptamine

The following procedure has been repeatedly followed after numerous trials using slightly different proportions or different conditions, and has been found to give the pure base in consistent yields.

A mixture of 80 gm. (0.5 mole) of γ-aminobutyracetal and 55 gm. of pure phenylhydrazine contained in a 1000-cc. round-bottomed flask is treated with 68 gm. of finely ground anhydrous zinc chloride. There is a moderate exothermal reaction and the mixture turns pale brown. An upright condenser is attached but no water is run into it. The flask is then gently heated by rotating it over a free flame and the greater portion of the alcohol formed in the reaction is distilled through the condenser. Further cautious heating is continued, preferably with a little local heating occasionally, until a rather vigorous exothermal reaction ensues. Water is then rapidly run into the condenser and the flask removed from the source of heat. When the reaction has subsided the fluid dark-brown mass is run onto the sides of the flask in order to present as great a surface as possible in the subsequent solution of the material. When sufficiently cooled, 60 cc. of acetic acid and 100 cc. of water is added and the mixture gently heated over a free flame until solution is complete. Water (about 600 cc.) is then added (the addition of the water precipitates a dark resinous material which need not be filtered off at this stage) and the zinc is precipitated with a stream of hydrogen sulphide. The zinc sulphide and resin are filtered off through a layer of charcoal and the pale yellow filtrate added to a concentrated solution of 100 gm. of sodium hydroxide. The oil which separates crystallizes on cooling or with great facility on seeding with a crystal of tryptamine. After remaining in the ice chest overnight the amine is filtered off, washed with cold water, and dried in a vacuum desiccator over potassium hydroxide. The yield of this product is 55 gm. (this and the subsequent yields are the average of four runs, the variation being less than

5%). The crude product is distilled in a vacuum, preferably of 2 mm. or less, from an oil bath and is thus obtained as a pale yellow viscous liquid which rapidly solidifies; yield, 47.4 gm. Further purification is conveniently effected by solution in the minimum volume of hot chloroform and slow cooling. There is thus obtained an almost colorless product consisting of stout polyhedra melting at 118°C.; yield, 41 gm. (51% of theory). The mother liquor on removal of the solvent and distillation of the residue yields a further small amount of equally pure product.

Acknowledgment

The author acknowledges his indebtedness to Dr. G. S. Whitby, Director of the Division of Industrial Chemistry, National Research Laboratories, for his criticism of the manuscript and permission to publish the same.

References

1. Asahina, Y. and Mayeda, S. J. Pharm. Soc. Japan, No. 416: 871. 1916.
2. Ewins, A. J. J. Chem. Soc. 99: 270-273. 1911.
3. Jackson, R. W. and Manske, R. H. F. J. Am. Chem. Soc. 52: 5029-5035. 1930.
4. Majima, R. and Hoshino, T. Ber. 58: 2042-2046. 1925.
5. Manske, R. H. F. Can. J. Research, 4: 275-282. 1931.
6. Power, F. B. and Salway, A. H. J. Chem. Soc. 99: 490-507. 1911.
7. Späth, E. and Lederer, E. Ber. 63: 2102-2111. 1930.
8. Wohl, A. Ber. 31: 1796-1801. 1898.

Canadian Journal *of* Research

Issued by The National Research Council *of* Canada

VOLUME 5 DECEMBER, 1931 NUMBER 6

VELOCITY OF LONGITUDINAL VIBRATION IN SOLID RODS (ULTRASONIC METHOD) WITH SPECIAL REFERENCE TO THE ELASTICITY OF ICE[1]

By R. W. Boyle[2] and D. O. Sproule[3]

Abstract

An experimental research showing how corrections in the value of velocity of phase propagation may be made to take into account lateral inertia, and how the law (Rayleigh's) will break down at higher frequencies because of other types of vibration intervening. When the ratio of radius to length, multiplied by the mode of vibration $\left(\frac{kr}{l}\right)$ exceeds a certain figure (in the case of duralumin, 0.55) the law breaks down, and it appears that radial longitudinal vibrations intervene. From the research a simple method emerges to determine Poisson's ratio, which in the case of duralumin is found to be 0.35.

An application of this ultrasonic method is made to determine Young's modulus for ice, in order to find more consistent values than those generally quoted. The value of this modulus for ice at about 0° C. is found to be about 9×10^{10} dynes/cm². corresponding to a velocity of sound in it of 3.15×10^5 cm. per sec.

Most measurements of the velocity of sound in a limited medium depend ultimately on interference phenomena in a reflected wave train. In the case of vibrating rods and bars the oscillation is generally adjusted to a condition of resonance and from the measurements of one or more half-wave-lengths, the velocity of phase propagation in the rod is deduced. For the natural modes of vibration (resonance) of a free rod, the length of the rod is equal to an integral number of half-wave-lengths, or $l = k\lambda$, where l is the length of the rod, λ is the wave-length, and $k = 1, 2, 3$, etc. corresponding to the mode of vibration. From the simple wave relation $v = n\lambda$, where v is the velocity and n the frequency, it follows that $v = \frac{2nl}{k}$.

The dimensions of a rod have a considerable influence on the half-wave-length in the rod for a given imposed frequency. In the case of a cylindrical rod, if the wave-length is large compared with the radius of the rod, or, what amounts to the same thing, if the ratio of the radius to the length, $\frac{r}{l}$, is

[1] *Manuscript received August 13, 1931.*

Contribution from National Research Laboratories, Ottawa, Canada. This paper was read before Section III of the Royal Society of Canada in May, 1931.

[2] *Director, Division of Physics, National Research Laboratories, Ottawa.*

[3] *1851 Exhibition Royal Commission Scholar from University of Alberta, Royal Institution, London.*

small, it is generally taken that $v = \frac{2nl}{k} = \sqrt{\frac{E}{\rho}}$, where E is Young's modulus of tensile elasticity and ρ is the density of the material. This is not strictly accurate, for the occurrence of Young's modulus connotes that the sides of the rod are not prevented from lateral bulging and shrinking, and bulging and shrinking involve a use of energy not accounted for in the formula. The value of the modulus employed should be the adiabatical or dynamic value and not the isothermal, which is generally determined by static or extensometer methods. In the case of solids, however, the difference between these two moduli is very small and is usually neglected.

For the gas column in a Kundt's tube $\frac{r}{l}$ generally has values of 0.005 to 0.1. For solid rods the simple wave relation above is often used with no particular regard to the important $\frac{r}{l}$ ratio. In the case of solids, for $\frac{r}{l} = \infty$, *i.e.*, for an infinite solid medium, in which the lateral bulging and shrinking which occurs in a rod is no longer possible, the velocity (V_0) is given by $V_0^2 = \frac{(1-\sigma)}{(1+\sigma)(1-2\sigma)} \frac{E}{\rho}$, where σ is Poisson's ratio.

It is impossible to perform sound experiments on "an infinite solid"—but a method to determine the velocity appropriate to the case emerges from the work of Boyle, Lehmann and Froman (2, 3). They found, by working with ultrasonic waves in water, incident perpendicularly on a partition of homogeneous material of thickness t, that the reflection was a maximum for t = an *integral odd number* of *quarter*-wave-lengths, and transmission a maximum for t = any *integral number* of *half*-wave-lengths. In some of the experiments the "partition" was merely a thin disk constituting the vane of a torsion pendulum by means of which the measurements were taken, in which case the vane itself was made to indicate the ratio of reflected or transmitted to incident energy. By plotting either the transmission or reflection ratio on the thickness, at a constant frequency of imposed waves, the wave-length in the material of the disk was found; the product of this wave-length with the frequency gives the velocity. Such velocities are always considerably greater than that computed for the material from quoted values of density and Young's modulus as determined by extensometer methods. For example, in duralumin, the velocity by the torsion pendulum ultrasonic method was found to be 6.48×10^5 cm. per sec., while from extensometer methods it was computed to be 5.16×10^5 cm. per sec. Such large divergences could not be explained by attributing them to experimental errors, and it is evident that assumptions made in applying the simple relation $V = \sqrt{\frac{E}{\rho}}$ are not satisfied in the conditions of the ultrasonic experiment. In the extensometer method $\frac{r}{l}$ is small, generally much less than 0.1, but in the ultrasonic method formerly described $\frac{r}{l}$ is relatively large, generally greater than 5. For example, in the case of the duralumin, Poisson's ratio $\sigma = 0.35$, the density $\rho = 2.79$, and $E = 7.5 \times 10^{11}$;

using the relation for an infinite solid, *viz*, $V_e^2 = \frac{(1-\sigma)}{(1+\sigma)(1-2\sigma)} \frac{E}{\rho}$ (1), it follows that $V_o = 6.57 \times 10^5$. This is in good agreement with the value 6.48×10^5 as determined with thin circular disks by the ultrasonic method. Thus it is indicated that with these thin disks the ratio $\frac{r}{l}$ is large enough to make the above formula approximately applicable, and that the velocity derived by this ultrasonic pendulum method is about the same as that deduced if the "elongational elasticity" (j) instead of Young's modulus is used in the simpler relation, $V_o = \sqrt{\frac{j}{\rho}}$ (1, pp. 125, 190).

Effect of Lateral Inertia

Early theoretical work on elastic vibration of a free cylindrical rod by Pochhammer (21) and Chree (7) was followed by that of Love (13, p. 289) and Rayleigh (23, pp. 251-253). Rayleigh's solution for longitudinal vibration was simple and practical and made a correction for the effect on the period of vibration of lateral inertia. The correction however took no account of any damping of vibration by solid viscosity or of the possible occurrence of resonant vibrations other than longitudinal lengthwise (such, for example, as radial longitudinal or torsional,) and it is on this account that the corrections will be incorrect after certain limits of frequency have been exceeded. The purpose of the present investigation was to demonstrate experimentally how these limits might be found.

The lowering of phase velocity on account of lateral inertia in a thick cylindrical rod may be deduced from the relation given by Rayleigh, as

$$V_2 = \frac{V_1}{1 + \frac{k^2\sigma^2\pi^2r^2}{4l^2}},$$

where V_1 is the velocity for a thin rod, *i.e.*, where $V_1 = \sqrt{\frac{E}{\rho}}$ may be safely applied, and V_2 is the velocity in a rod of significant radius (r) as compared with the length (l); k is the integer, 1, 2, 3, etc., representing the particular mode of vibration.

For a fixed length of rod, if V_2 were constant for all longitudinal resonances the frequencies of successive modes would be in the ratio 1, 2, 3, etc., that is, the overtones series would be harmonic. But a change in V_2 with successive modes, as is indicated by Rayleigh's expression, results in departure from the harmonic series. The phase velocity V_2 according to this expression is a function of $(\frac{kr}{l})$, *i.e.*, depends on the radius and length of the rod, and on the mode of vibration.

Rayleigh mentioned the improbability of confirming experimentally his correction for lateral inertia because of the difficulty of experiment then existing with rods short enough and thick enough to make the correction appreciable; but since the advent of ultrasonic oscillators, operated by adjustable frequency oscillating electrical circuits, this difficulty disappears. During the Great

War experimenters in England investigated to some extent the longitudinal oscillations in steel rods excited electromagnetically in the phenomenon of magneto-striction, but no results were published. More recently, Quimby (22) and also the present writers (5) experimented with metal rods excited into oscillation by the piezo-electric effect of quartz. Pierce (20) and also Muzzey (17) determined elastic constants and velocities with high precision in rods or tubes of certain ferromagnetic metals by Pierce's high frequency magneto-striction oscillators. By this method, Muzzey confirmed Rayleigh's formula for small values of $\frac{kr}{l}$ in the case of stainless steel. By a very interesting method of substitution Klein and Hershberger (10) measured the longitudinal velocity in a slab of solid with their "supersonic interferometer". Setting up in the interferometer a stationary wave system in a column of liquid in which the velocity is known, by inserting a parallel faced slab of solid in the liquid path, the nodal planes are displaced and the displacement measured. The difference in *acoustical path* with the solid absent and then present could thereby be determined and the velocity in the solid deduced.

The present work by the authors was carried out four years ago, and though the determinations claim no high degree of precision it was thought advisable to put them on record. The paper is an amplification of results and indication of the method which already have been briefly communicated (5).

Experimental

Cylindrical metal rods, A, of uniform diameter and equal length were cemented to each side of a quartz disk, B, so that the rods were coaxial, as is shown in Fig. 1. Terminals from a valve oscillator were connected to the metal rods. With this arrangement the even numbered modes of vibration were quite weak, and the numerical results here shown were obtained from the odd numbered modes only.

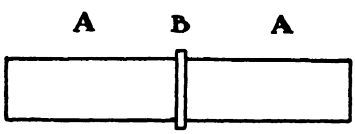


FIG. 1.

Under the above conditions the oscillating electric field imposed on the quartz sets up by piezo-electric action a pressure wave which travels out into the metal rods. When the frequency of the electric field corresponds to the natural frequency of one of the modes of mechanical vibration of the quartz-metal combination, the intensity of the mechanical vibration is increased greatly due to resonance. This increase may be detected by any one of several methods as will be indicated later. In no experimental case was the wave-length in the solid rod so short that a sharp ultrasonic "beam" could be emitted from the quartz; for such a condition the diameter of the rod would have to be many times a wave-length. The reverse was the case.

Other arrangements than the one described were used to start the vibrations. Various combinations of long and short rods using active quartz were possible, even to placing a piece of quartz at one end only. The method of placing a strip of quartz on the side of a rod gave comparatively feeble oscillations. In addition iron rods were made to vibrate by the direct magneto-strictive action of the alternating fields of magnetic force near the oscillating coils.

The chief objection to using a composite rod composed of a quartz layer and lengths of another material is that an appreciable correction may become necessary if the velocity of sound in the quartz is not the same as the velocity of sound in the experimental material. The correction may be found as follows: The necessary condition for resonance at the fundamental frequency is that the sound travels the whole length in half a period. Let l = length of rod, d = thickness of quartz, V_q = velocity of sound in quartz, V_m = velocity of sound in the metal pieces, n = frequency and T = period $\frac{1}{n}$. The time required for sound to travel through the metal is $\frac{l-d}{V_m}$ and the time required for the sound to travel through the quartz is $\frac{d}{V_q}$. Hence $\frac{l-d}{V_m}+\frac{d}{V_q}=\frac{1}{2}T=\frac{1}{2n}$, whence $V_m=\frac{(l-d)\,2nV_q}{V_q-2nd}$. Taking $V_q=5.5+10^5$, and $d=0.2$ cm., it was found that the calculated correction for the rods used was in all cases less than 1%, and therefore not more than the variation in velocity from sample to sample of material used for the rods. Hence its application was unnecessary within the limit of accuracy permitted by these experiments.

The method of experiment was to set the oscillator vibrating, carefully adjust the electrical frequency until a resonant longitudinal mechanical vibration of the oscillator could be detected, then measure the electrical frequency with a calibrated Hertzian wave meter. The method suitable for the detection of the resonant longitudinal vibration depended somewhat on the power and frequencies employed in the experiment. The following devices were successfully employed at various times, the particular one selected depending on the circumstances of the work. Usually the observed oscillations were generated by a 250-watt valve oscillator set. Experiments of the same kind as are described here have been generally carried out by other workers at much weaker energies.

(1) By imposing rectified 60 cycles per sec., or higher periodicity, alternating potential on the plate of the valve oscillator a "tonic-train" oscillation was made to modulate the high frequency mechanical vibrations of the rod. A stethoscope with a cup-shaped soft rubber "listening" tip could be applied to one of the vibrating ends of the rod to detect the "tonic-train" when the rod vibrated at or near its resonant frequencies. A stethoscope with double tip, as shown in Fig. 2 which is self-explanatory, was devised to utilize in detection the sound energy from both ends of the oscillating rod. The same results were obtained using stethoscopes with either a single or double tip.

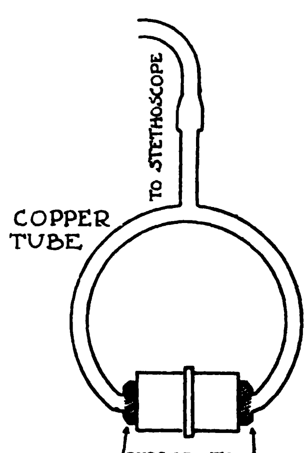


FIG. 2.

(2) By placing one end of the vibrating rod in a suitable liquid, cavitation (bubbling) of the liquid could be produced at some of the resonant frequencies of the rod (6). Bubbles of dissolved gas could be

driven even from ordinary water, thus indicating that in some cases the energy intensity emitted from these small resonators could sometimes exceed the intensity in beams from much larger transmitters.

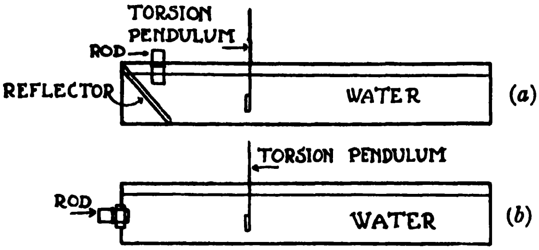


FIG. 3.

(3) As indicated in Fig. 3, (*a* and *b*), the ultrasonic beam emitted from the end of the rod at resonance could be used to detect some of the resonant frequencies, the indicator being the deflection of a torsion pendulum suspended in a suitable liquid in a small tank.

(4) By the use as detector of an ordinary coupled low power radio receiving set, in a variety of ways such as follows— (a) The simple procedure often used in experiments with piezo-electrically controlled radio oscillators—as the frequency of the rod oscillator is adjusted to pass through a longitudinal resonance, a sharp "chirp" is heard in the telephones of the coupled radio circuit. This useful phenomenon is due to the fact that the oscillating rod takes extra energy from its driving circuit when the frequency (f_1) of that circuit is the resonant frequency of the rod; but on slightly changing the frequency of the driving circuit to f_2, the rod feeds the energy back into the circuit at the frequency f_1, so producing an audible beat or "chirp" in the phones for values of f_2 near f_1. (b) The fact that the oscillating rod takes extra energy from its driving circuit at the frequency of resonance may be shown by a "kick" of a galvanometer of requisite sensibility placed in the oscillator plate circuit, as indicated in Fig. 4. (c) The rods made to vibrate by mechanical instead of electrical means by tapping them sharply at one end with a hammer. This method can be used only up to certain limits of frequency (12). A coupled radio receiving set can detect the resonances in (4a) above.

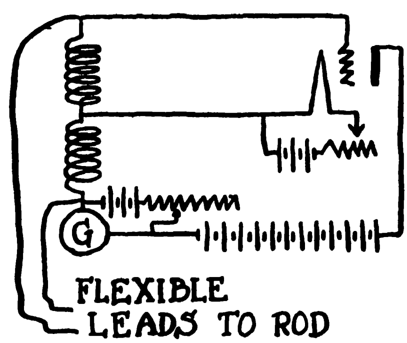


FIG. 4.

(5) By supporting the oscillating rod vertically and placing light particles, such as powdered cinders or small glass beads, on the upper end, resonant frequencies can be detected by the shifting and movement of the particles at these frequencies. The motions of these particles were found to yield an insight into the possible complex vibrations in the rod, especially at the higher frequencies, as discussed later (Fig. 8).

(6) Some of the experiments were carried out when the rod was oscillating in the audio-frequency range, in which cases the simplest method of detecting resonance was to adjust the frequency until the maximum audibility of emitted sound was heard. Some of the other methods enumerated above could also be used in this case.

Whatever the method employed to detect the resonances, great care must be exercised by checking and re-checking between the methods to be certain that it is a *longitudinal resonance* and not resonance of another type of vibration which is observed, otherwise the calculations for velocity have no validity. This important precaution may easily be overlooked.

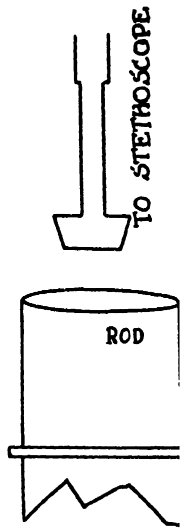


FIG. 5.

[Note: In working with detection method described above, in many cases the note of the tonic train becomes inaudible or nearly so when the listening tip of the stethoscope is lifted barely clear of the vibrating surface (Fig. 5) but becomes audible at a point further away. Calculation revealed the fact that the distance from the listener to the end of the oscillating rod was half a wave-length of the emitted ultrasonic waves in the air. By employing rods of greater than the usual diameter, and increasing the effective reflecting surface of the listener, it was found possible in some cases to detect nodes and antinodes in the air for as many as 100 successive half-wave-lengths Knowing the value of the velocity of sound in air it was possible to determine the frequency of the oscillator by this method; or, on taking the Hertzian wave-meter frequencies as standard, a check of better than 1% in the velocity of sound in air was easily obtained this way. The simplest method of obtaining a large reflecting surface for the listener was to stretch a very thin rubber diaphragm over the mouth of a thistle funnel as shown in Fig. 6. This experiment is of the same type as that described by Pierce (19) and by Hubbard and Loomis (9), who determined the nodal positions of the reflector by the reaction of the reflected ultrasonic wave on the electrical circuit. In the present case the nodal positions were determined by the reactions of the wave on the reflector itself, through the audibility of the tonic train.]

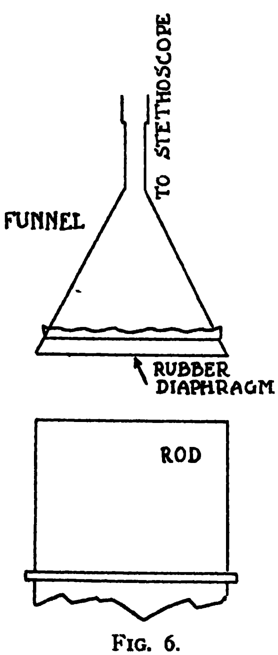


FIG. 6.

Results

The first rod used was designed so that the correction for lateral inertia would not be considerable for its first (or fundamental) mode of vibration, but would be appreciable for the higher modes. Duralumin was selected as a suitable material. Two cylinders of duralumin 7.0 cm. long and 5.1 cm. in diameter were turned on the lathe. These were cemented coaxially to the opposite faces of a piezo-electric quartz disk 0.19 cm. thick, thus making a composite rod 14.19 cm. long. The velocity of sound in this rod at room temperature as determined from $V=\sqrt{\frac{E}{\rho}}$, using the known values of density and Young's

modulus, *viz.* 2.79 and 7.4×10^{11} respectively, was 5.16×10^5 cm. per sec.; the velocity in this rod determined experimentally by the ultrasonic methods here described was 5.08 cm. per sec.

A long series of experiments employing many rods of different proportions was now carried out. The rods were all carefully machined from the same large piece of duralumin, most of them being shortened lengths of the longer rods and smaller diameter pieces of the thicker rods. The temperatures were always around the ordinary room temperature of 18° C.

In some of the rods the diminution of velocity by lateral inertia was appreciable even for the first mode of vibration, in others the diminution was inappreciable up to the seventh mode. In Table I are assembled the numerical results.

TABLE I

EFFECT OF DIMENSIONAL CHANGES ON VELOCITY

r	l	k	kr/l	Velocity, cm./sec., calc. from Rayleigh's formula	Frequency in cycles/sec.	Velocity, cm. sec., from experiment
0.63	14.2	1	0.0444	5.10×10^5	17900	5.09×10^5
		3	0.133	5.08	53500	5.07
		5	0.222	5.03	88300	5.01
0.95	14.2	1	0.0668	5.10	17900	5.09
		3	0.220	5.04	53200	5.04
		5	0.334	4.93	88000	5.00
0.95	61.2	3	0.0466	5.10	12520	5.11
		5	0.0776	5.09	20900	5.12
		7	0.109	5.08	29200	5.11
		9	0.140	5.07	37350	5.07
		11	0.171	5.05	45500	5.06
		13	0.202	5.04	53600	5.05
1.25	6.21	1	0.201	5.04	40600	5.05
1.25	12.2	1	0.103	5.08	20700	5.05
		3	0.307	4.96	61000	4.96
		5	0.513	4.72	94000	4.59
1.25	14.2	1	0.0880	5.08	17900	5.08
		3	0.264	4.99	52700	4.99
		5	0.440	4.81	83800	4.76
2.55	8.25	1	0.309	4.96	30000	4.95
2.55	14.2	1	0.179	5.05	17900	5.08
		3	0.539	4.68	48500	4.60
		5	0.898	4.18	61500	3.50

From the table and curve, Fig. 7, it may be seen that with duralumin for $\frac{kr}{l} < 0.1$ the effect of lateral inertia is not very appreciable. In the range $\frac{kr}{l} > 0.1$ and < 0.55 Rayleigh's expression gives the velocity closely enough for most purposes. For $\frac{kr}{l} > 0.55$ the experiments demonstrated that the

modes of vibration are more complex, and that it is virtually impossible to obtain trustworthy observations in the experiments.

A study of the behavior of dust particles on the vibrating end surface of the rod oscillator throws additional light on what is taking place. Dust of sifted cinders or duralumin filings proved suitable for this use. For the modes of vibration giving numerical results consistent, as above, with Rayleigh's correction, the dust particles were simply agitated up and down on the end of the rod without showing any tendency to arrange themselves in any particular pattern on the vibrating end surface; this might be expected for a pure longitudinal oscillation. But for vibrations when $\frac{kr}{l} > 0.5$ a distinctly different type of behavior was observed. At certain frequencies, for example between 84000 and 300,000 cycles/sec. for a rod 48 cm. long and 5.1 cm. diameter, the dust arranged itself in various patterns. Four-, six-, eight- and twelve-pointed stars could be obtained. (The photograph shown in Fig. 8 represents an eight-pointed star.) Some sort of radial vibration must be assumed to account for such figures of radial symmetry.

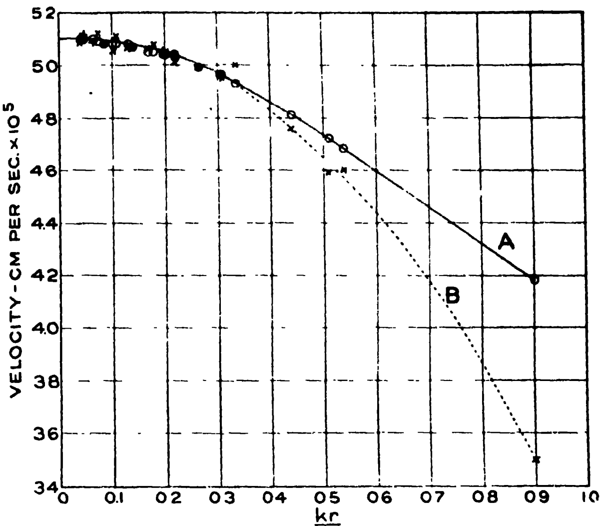


FIG. 7.

Another type of behavior of the particles was even more striking. At certain frequencies the particles were observed to move continuously in a circle about the centre of the rod. For others, the particles near the outer edge of the end of the oscillator moved in a clockwise direction, while those near the centre moved in the counter direction. At times, little whirls formed off to one side of the centre.

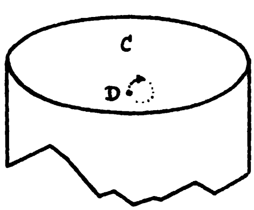


FIG. 9.

A combined torsional and longitudinal vibration might account for these circular motions, if the two forms of vibration had a fixed phase relation. For example, consider a small element, D, of the surface at one side of the centre, C, Fig. 9. If the rod is executing combined torsional and longitudinal vibrations in an appropriate phase relation, then at a given instant the point D may be moving up due to the longitudinal vibration and, at the same time, beginning to move to the right due to the torsional vibration. A quarter period later it would be beginning to move down due to the longitudinal vibration and would be moving to

the right due to the torsional vibration. It can be seen that in such a case the particle D would be shot to the right once every cycle. If all parts of the surface equidistant from the centre were vibrating in the same manner, every surface particle on this ring would be shifted in the same manner and would behave as the particles are observed to do. Harmonics of the torsional vibration would explain the existence of more than one circle of particles as were sometimes observed.

The intricate complexities of vibration, which may exist at high radio-frequencies in the quartz blocks and rods used in radio frequency stabilization have recently been demonstrated by similar dust figure methods to the above.

It is clear that the experiments warn against any idea that a thick rod will always vibrate in a longitudinal manner because it is supplied with longitudinal vibrating energy. It is certain that types other than longitudinal vibration will take place. Even in thin rods more than one type of vibration may exist, though this is not readily observable; but as the rod thickens the distribution of the energy into more than one dominant type may, by their resonances, become more easily observable. In reality a rod, especially a thick one, when supplied with any one type of vibrational energy may become a vibrating system of almost all other types.

Incidental

(1) *Audible Beat Notes*

During the above experiments an interesting fact was sometimes observed. An audible note could be emitted from the thick rods when they came into resonance, even when the driving electrical oscillations were at the ultra-audible frequency of 100,000 cycles per sec. or more. This is readily explained by the fact that in the successive modes of vibration of a rod, beats may be caused between two or more of the overtones. If the beat frequency is lower than about 20000 cycles per second, the beat note will be audible, provided the intensity is sufficient. This was often the case.

(2) *Determination of Young's Modulus for Metals*

As an application of the methods described here, determinations were also made of the velocities in rods of iron, brass, magnesium, and type metal. Results for these rods are compared with those computed from values of Young's modulus and density and are shown in Table II.

TABLE II

COMPARISON OF VELOCITIES OF LONGITUDINAL VIBRATION IN METAL RODS OBTAINED BY THE ULTRASONIC METHOD WITH THOSE COMPUTED FROM VALUES OF YOUNG'S MODULUS

Material	Velocity by ultrasonic method, cm. per sec.	Velocity from $V=\sqrt{\frac{E}{\rho}}$, cm. per sec.	Material	Velocity by ultrasonic method, cm. per sec.	Velocity from $V=\sqrt{\frac{E}{\rho}}$, cm. per sec.
Iron	4.95×10^5	4.99×10^5	Magnesium	5.02×10^5	4.98×10^5
Brass	3.47×10^5	3.50×10^5	Type metal	1.71×10^5	1.72×10^5

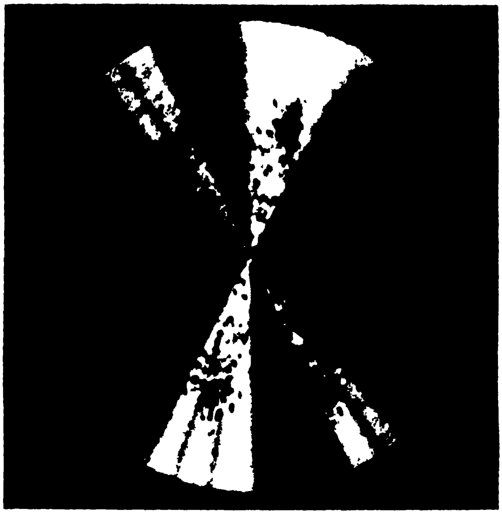


FIG. 8

(3) *Determination of Compressional Elasticity of Non-metallic Solids*

As a further application the compressional elasticity of a non-metallic solid, *e.g.*, marble, was determined.

Two rectangular prisms of marble were sealed to tin-foil electrodes which in turn were sealed to opposite faces of the quartz—to compose an ultrasonic oscillator of marble. Fig. 10 represents the arrangement. The marble prisms were 8 cm. long with sections 2 by 4 cm. Velocity so measured was 4.06×10^5 cm. per sec. A quoted value gives the velocity 3.8×10^5; it is likely that the determination by the ultrasonic method is the more correct.

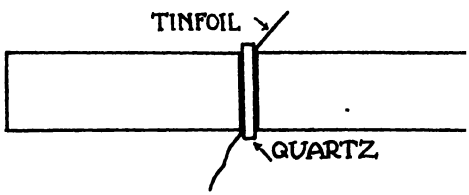


FIG. 10.

Discussion

(a) The longitudinal velocity in a "thin" cylindrical metal rod is given by $V_1 = \sqrt{\frac{E}{\rho}}$, if a "thin" rod is defined as one for which Rayleigh's correction for lateral inertia is inappreciable. Young's modulus as determined by longitudinal vibrations in such a rod will be, within the experimental error, the same as the modulus obtained by static loading, although the stresses set up in these two cases are so different Researches of Swift (24) and of Pichot (18) have already shown this to be the case for flexural vibrations in rods of metal and stone This agreement might be expected as the stresses in the two cases are identical in type, both taking place in the direction of the long axis of the rod. However, a discussion of possible differences between the dynamic and static methods in other cases is included later in the paper in the section concerning the work on ice.

(b) The formula for the velocity of sound in an infinite solid may be written $V_o^2 = \frac{(1-\sigma)}{(1-\sigma)(1-2\sigma)} \cdot V_1^2$. V_o may be measured by the method employed by Boyle and Lehmann (2), while V_1 may be determined by the method described here. Hence, σ is determined. This result is of special interest as Poisson's ratio is a most difficult constant to determine accurately by direct experiment. For duralumin, by the method here, σ is computed to be 0.35.

(c) Also, σ may be obtained from the expression for (V_2), since $\sigma = \frac{(V_1 - V_2)4l^2}{k^2\pi^2r^2V_2}$ where V_2 = vel. in thick rod, and V_1 = vel. in thin rod. In this case V_1 may be determined from the first mode of vibration and V_2 from any succeeding mode by using a rod of suitable dimensions within the range of applicability of Rayleigh's formula. This method has the advantage of permitting Poisson's ratio to be measured from simple observations on the same small piece of material.

(d) In Muzzey's experiments (17) on stainless steel by the magneto-strictive method a range of $\frac{kr}{l}$ from zero up to a value of 0.25 was covered and good agreement with Rayleigh's formula was shown. The results of the present

experiments on duralumin within the same range of $\frac{kr}{l}$ agree with this conclusion, and find that $\frac{kr}{l}$ has to reach a value of about 0.55, in the case of duralumin, before the longitudinal velocity diminishes by more than 2%. It is at higher values of $\frac{kr}{l}$ in this material that the law more seriously breaks down, for the chief reason that resonances of other types of vibration are beginning to intervene. Possibly also viscosity under these conditions is having a greater effect. Other materials will have a corresponding set of values of $\frac{kr}{l}$ which will determine the types and modes of vibration that are possible.

(e) It should be noticed that in the present experiments detection of the longitudinal resonances of the rods was made sometimes by mechanical means, such as "listening" devices, torsion pendula and cavitation, and sometimes by electrical reactive methods like telephones or galvanometers acting reactively in the electrical oscillating circuit. No differences in the resonant frequencies could be found when using mechanical or reactive detectors. When a reactive electrical detector is used certain considerations must enter into the interpretation of the value of frequency to which the detector makes its maximum response. In the case of magneto-striction it can be seen from Pierce's (20) theory of the oscillator, and as pointed out by Muzzey (17), that the equations of motion for the mechanical vibrations in the rod should include terms to represent (a) the forcing of the rod by magneto-striction and (b) the magnetic reaction of the vibrations of the rod on the magnetic induction through the coils. On solving the complete equation for the resonant frequencies (which will be the frequencies indicated by a reactive detector) the same form of relation for frequency will be found as when these extra terms were omitted, but the "constant" representing the elasticity E is now increased by a small amount, (which is the product of two coefficients, one of them mechanical, the other magnetic).

Similar considerations must apply in the piezo-electric oscillator—the extra terms in the equation of motion will represent (a) the forcing of the rod by piezo-electric action and (b) the electrical reaction of the vibration of the rod as a back e.m.f. in the driving circuit. In the formula for resonant frequency (as detected by a reactive detector) the constant E will be increased slightly. This consideration would not apply in the case of a mechanical detector outside the driving electrical circuits.

As mentioned above, in the experiments on duralumin no differences in resonant frequencies were detected when using mechanical and reactive detectors, consequently the product of the two coefficients in the piezo-electric case must be small and negligible in comparison with E. Muzzey's experiment showed that in the case of magneto-striction oscillators of stainless steel, the value of E_1 came out to be about the same value as would be expected for Young's modulus, that is, the product of the two coefficients is negligible in comparison with E. This product in the piezo-electric case is likely to be even smaller than that in the magneto-strictive.

Part II. Special Reference to the Elasticity of Ice

Young's Modulus for Ice

In association with work on iceberg detection by means of an ultrasonic beam, carried out by Boyle and Reid (4) in the Gulf of St. Lawrence, 1924, it became expedient to apply the method described here to the determination of the elastic constant of ice. Investigations to determine Young's modulus for ice had been carried out before by various workers employing static bending methods, but exceedingly discordant results have been obtained.

A glance at Table III reveals that this modulus for ice either varies a great deal from sample to sample, or else something is very uncertain in the static methods of experiment with this material. The application of the methods of this paper indicates a small variation from sample to sample of ice but nothing of the order indicated in Table III.

TABLE III
YOUNG'S MODULUS FOR ICE

Observer	Young's modulus kg. cm.[2]	Observer	Young's modulus kg. cm.[2]
Moseley	92700	Hess	27600
Benan	60000	Weinberg	50000
Renach	23630		

A further lack of agreement is to be found on examining the results of investigators who were searching for a difference in Young's modulus in directions parallel and perpendicular to the optic axis. Some claim no appreciable difference, others large differences of the order indicated by the results of Matsuyama (14). By the bending method Matsuyama found that Young's modulus for the bar of ice, cut so that the optic axis was perpendicular to the long axis of the bar and also to the plane of bending, was 9400 kg.cm.$^{-2}$*; a bar cut so that the optic axis was parallel to the plane of bending and also to the long axis, gave a modulus of 18900 kg.cm.$^{-2}$, while one with the optic axis parallel to the plane of bending but perpendicular to the long axis yielded a modulus of 6100 kg.cm.$^{-2}$. As will be seen later Young's modulus for ice as determined by ultrasonic longitudinal vibrations indicates that any variation of the modulus with respect to direction in the ice crystal is not great.

[Note. A more accurate determination of Young's modulus for ice is of incidental interest on another account. Thornton (25) pointed out the possibility of a simple relation existing between the thermal conductivity, Young's modulus, and the density, of solid non-metallic insulators. It appeared that the expression $K=E\rho=V^2\rho^2$, (order being omitted) where K=heat conductivity, might be valid generally. In computations from tabulated constants Thornton found that this relation held in a fair number of cases. Clarke (8) pointed out that this relation did not hold for a very homogene-

* *In the original paper there appears to be an error in the naming of the units expressing the results.*

ous insulator like an optical glass and thought that the agreement shown by the materials chosen by Thornton was apparently a matter of chance. Thornton's relation for ice could be checked if some indication could be obtained as to which of the many quoted values of Young's modulus was correct. Taking the value of E for a sample of ice at 20° C. as found in this research (p. 616) as 9×10^{10}, and ρ as 0.92, the product $E\rho$ is 8.3×10^{10}. The quoted value of heat conductivity for ice (K) is 0.051. The suggested relation $K=E\rho=V^2\rho^2$ (order being omitted) therefore does not even approximately hold.]

From the foregoing experiments of Part I on metal rods it can be seen that a simple, rapid and reasonably accurate dynamic method is available for finding Young's modulus of any solid. Two advantages in particular are associated with the method of high frequencies, namely, a small sample of the experimental material is sufficient and more convenient than a large one, and the frequency is readily measurable to considerable accuracy. Hence, it may be considered that values of the elastic constant for ice determined by this method are more dependable than those from former determinations.

In making the ultrasonic ice oscillators, owing to the fact that ice is a good electrical insulator, it was necessary to cement tinfoil to each side of the active piezo-electric quartz with sealing or other wax before freezing the ice rods to the quartz disk, as indicated in Fig. 10. The samples, rectangular prisms, were first cut roughly from the ice block with a metal saw, and then shaped more exactly by the melting of their surfaces as and where required on a slightly warmed flat metal plate. The sections of the prisms were about 2 cm. square, and smaller sections gave the same results. Since no suitable methods of artificial refrigeration were available, the experiments were performed out of doors, during the months of November, December and January, 1927-28. The rods of ice were placed outside a window of the experimental room at times when the temperature was suitable.

It cannot be said with exactness that the ice samples employed here were cut from a single ice crystal, for such are often difficult to obtain in sufficient size, and twinning is very common. The samples were cut from perfectly clear blocks of ice taken from a river where freezing had taken place slowly in relatively quiet and unagitated water. Although not invariably true, generally it is the case that when freezing takes place slowly in still water, the ice crystals form with the optic axis in or very near the vertical. Hence, in the present experiments, it was considered that the direction of the optic axis of the samples was in or near the direction of the vertical in the naturally occurring block of ice.

Effect of Temperature

The variation with temperature of Young's modulus for ice was investigated as well as possible under the local conditions from 0° to −35° C. This was done by taking observations on the exposed ice oscillators as the outside air temperatures changed as noted. Results are given in Table IV. A small increase in E with a decrease in temperature was found. Here the inability to

control the temperature was a handicap for a time lag in the value of E behind the temperature was noticed. The heat conductivity of ice is low, but if this lag had been due only to the time required for the ice to take up the temperature of the surroundings, the lag would have been greater for the larger rods. This was not the case. The effect might be worth studying in a laboratory where low temperature facilities are available.

TABLE IV

VARIATION WITH TEMPERATURE OF YOUNG'S MODULUS FOR ICE. SAMPLE WITH OPTIC AXIS PARALLEL TO THE LONG AXIS OF THE ROD

l, cm.	Temp., °C.	Frequency, cycles per sec.	Velocity, cm. per sec.	E, dynes per cm.^{2}
12.3	-9.0	12930	3.18×10^5	9.29×10^{10}
11.9	-10.0	13530	3.21×10^5	9.48×10^{10}
11.9	-30.0	14000	3.33×10^5	$10.2\ \times10^{10}$
12.3	-35.0	13950	3.43×10^5	$10.9\ \times10^{10}$
11.9*	At some temp. between $-30°$ and $-60°$ C.	14880	3.54×10^5	$11.5\ \times10^{10}$

**The last result quoted in the table came from an attempt to extend the temperature range downward by placing the ice oscillator in a thermos bottle and cooling with "carbon-dioxide snow". Several readings were taken as the ice cooled. But due to the unequal contraction of the ice and quartz, the rods fell apart at some temperature between $-30°$ and $-60°$ C. and the experiment could not be repeated.*

A qualitative idea of the effect of temperature on the solid viscosity and damping of vibration in the ice was incidentally observed from the intensities of the emitted note when the rods oscillated at audio-frequencies. On cold days (temperature about $-30°$ C.) the note of the resonant ice rods was plainly audible at a distance, but on relatively warm days the sound was barely audible even close to the oscillator.

The results with ice by this method cannot be as accurate as with other solids for a number of reasons. One is that the material does not lend itself to the work so readily. Other reasons will be discussed later. It was found that there were variations in E from 9.0×10^{10} to 10.5×10^{10} even with rod oscillators cut from the same block of ice and in the same direction—changes of temperature of course explaining some of these differences.

Effect of Direction in the Ice Crystal

In order to determine the possible effect of orientation of the optic axis with respect to the long axis of the rod oscillator, many rod samples were cut from the same solid block of ice. In some samples the long axis of the rod was parallel to, in others perpendicular to, and in others inclined at about 45° to, the vertical in the ice as frozen. Results are given in Table V

A glance at Table V reveals the fact that any variation there may be in E in different directions in the ice crystal is slight in comparison with the great variation quoted from other investigations, and not greater than the variation from sample to sample of ice cut in the same direction from the same block. This is not equivalent to saying there is no variation in different directions

TABLE V

RESULTS OBTAINED WITH ICE SAMPLES CUT IN DIFFERENT DIRECTIONS

Direction of cut	l, cm.	Temp., °C.	Frequency, cycles/sec.	Velocity, cm./sec.	E, dynes/cm.^{2}
Cut parallel to the vertical	13.6 13.5 13.5	−20 −26 −33	11600 11900 12000	3.15×10^5 3.24 3.26	9.12×10^{10} 9.67 9.75
A. Cut perp. to vertical	20.85 20.7 20.7	−20 −26 −33	7600 7840 7900	3.17 3.27 3.29	9.2 9.85 10.0
B. Also perp. to the vertical —but in the 3rd rectilinear direction	20.6 20.5 20.5	−20 −26 −33	7620 7770 7800	3.14 3.19 3.20	9.06 9.37 9.40
Cut at 45° to the vertical	21.8 21.7 21.7	−20 −26 −33	7040 7150 7150	3.07 3.11 3.11	8.65 8.92 8.92

greater than the experimental error. The variation from sample to sample is probably not due to experimental error, but to differences in the elastic nature of the ice introduced sometime during its past history. Probably some differences in E in different directions will exist even if the rods are prepared under uniform conditions and preserved at a uniform temperature during the course of the experiment.

Discussion

A general statement by Swift (24) on the nature of elasticity, together with the work of McConnel and Kidd (15, 16) on the elasticity of ice, may adequately account for the variations shown in values of the elastic constant as given here.

According to Swift, "the deformation, which follows a change of static loading may, in the most general cases, be resolved into three separate constituents. (*a*) The strain which develops simultaneously with the change in stress, and of which it may be regarded as the cause or effect. This strain is essentially reversible in nature. (*b*) A deformation essentially permanent and irreversiblewhich develops more slowly and with the passage of time approaches (in an irregular but roughly logarithmic way) its final value. (*c*) A strain which develops slowly in a similar way to the permanent set, but which is reversible. This strain is made evident in the partial recovery ("elastic afterstrain") which follows the removal of load from so-called semi-elastic materials, or from materials which have been overstrained, and in the reversible creep which occurs with elastic materials at high temperatures even under stresses which have apparently no permanent residual effect.

The processes which give rise to these changes are not known, but from the simple fact that strain may have this composite nature it follows that unless some regard is paid to the factor of time in the definition, the modulus of elasticity has no real physical significance and a restricted practical value."

The work of McConnel and Kidd indicates most clearly that in the case of

ice, the components (*b*) and (*c*) are ordinarily large parts of the whole strain, and may both be greater than (a); and that not only are (*b*) and (*c*) dependent on time, but also on temperature. Investigators determining the elastic modulus of ice probably were unaware of these facts and since no precautions seem to have been taken to avoid or make known these last two components agreement is not to be expected.

Again the work of McConnel and Kidd indicates a possible reason for the discrepancy between Matsuyama's results and those obtained in this investigation. They found that component (*b*) mentioned by Swift, varied with the manner of cutting the sample of ice with respect to the optic axis. Hence, unless precautions are taken to separate component (*b*) in each case the different determinations of E are not comparable.

The work of Swift with flexural vibrations in metal rods, and also the work of Pichot (18) on bars of rock, indicate that a dynamic method of determining E effectively eliminated factors (*b*) and (*c*). As has already been suggested, there is reason to believe that these general conclusions will be the same for longitudinal vibrations, for the stresses and strains take place in the direction of the long axis of the rod in both cases.

In brief, the work of McConnel and Kidd indicates that the weakness in a static method of elasticity determination applied to ice is that the components of deformation which are functions of time cannot be eliminated or made known. The work of Swift and Pichot, on the other hand, shows that by dynamic methods the components of deformation involving a time element may be eliminated and at the same time fairly accurate determination of the physical value of Young's modulus may readily be made.

As already mentioned, it was found in the present work on ice that there could be variations in E, at the same temperature, from sample to sample varying from 10.5×10^{10} to 9.0×10^{10} dynes/cm^2., even with rods cut from the same block of ice and in the same direction. But the differences in the values of E observed here are small in comparison with those obtained by static methods, though they are large in comparison with the experimental error of the present method and therefore cannot be so ascribed.

In this ultrasonic method the possible sources of error are:— (1) Error in measurement of the length of the ice rods; and (2) error due to possibly mistaking the identity of one mode or type of vibration for a totally different mode or type, *e.g.*, assuming that the fifth mode of transverse vibration is the first mode of longitudinal vibration; and (3) error in measurement of frequency. The errors in (1) and (3) together were not over 1%. An error due to a mistake in identity of one mode of vibration for another would result in value of velocity which was in error by some simple multiple of the true velocity. The transverse types of vibration were easily distinguished from the longitudinal types by the use of the stethoscope, for placing a stethoscope tip at the side of a bar when it was vibrating in a longitudinal fashion resulted in a sound very faint in comparison with that obtained by placing the tip at the end of the same vibrating rod. For the case of a transverse vibration the contrary was the case.

Hence the variation in velocity from sample to sample of ice of such magnitude as was shown here must be due to a real variation in the elastic properties of the samples. The discordant results quoted by Wyckoff (26) from the X-ray analysis of ice indicate that the crystal structure of ice may well be a function of its past history. A change in crystal structure in general must lead to a change in the elastic properties of the crystal, and though the continuous deformation of ice under constant stress observed by McConnel and Kidd also indicates that the crystal structure of river ice might be expected to be irregular, the results of this research indicate that the large differences in values of elastic constants formerly quoted cannot be considered correct.

References

1. Barton, E. H. Text book on sound, 2nd ed. Macmillan. 1908.
2. Boyle, R. W. and Lehmann, J. F. Trans. Roy. Soc. Can. 111, 16: 115-125. 1927.
3. Boyle, R. W. and Froman, D. K. Can. J. Research, 1: 405-424. 1929.
4. Boyle, R. W. and Reid, C. D. Trans. Roy. Soc. Can. 111, 20: 233-243. 1926.
5. Boyle, R. W. and Sproule, D. W. Nature, 123: 13. 1929.
6. Boyle, R. W., Taylor, G. B. and Froman, D. K. Trans. Roy. Soc. Can. 111, 23: 187-201. 1929.
7. Chree, C. Quart. J. Math. 21. 1886.
8. Clarke, J. R. Phil. Mag. 4: 502-504. 1920.
9. Hubbard, J. C. and Loomis, A. L. Nature, 120: 189. 1927.
10. Klein, E. and Hershberger, W. D. Phys. Rev. 37: 760-774. 1931.
11. Lamb, H. Dynamical theory of sound, 2nd ed. Longmans. 1925.
12. Lang, R. J. Trans. Roy. Soc. Can. 111, 16: 163-173. 1922.
13. Love, A. G. H. Mathematical theory of electricity, 4th ed. Camb. Univ. Press. 1927.
14. Matsuyama, M. J. Geol. 28: 607-631. 1920.
15. McConnel, J. C. Proc. Roy. Soc. 49: 323-343. 1891.
16. McConnel, J. C. and Kidd, D. A. Proc. Roy. Soc. 44: 331-367. 1888.
17. Muzzey, D. S. Phys. Rev. 36: 935-947. 1930.
18. Pichot, M. J. Phys. radium, 6 ser. 8: 422-432. 1927.
19. Pierce, G. W. Proc. Am. Acad. Sci. 60: 271-302. 1925.
20. Pierce, G. W. Proc. Am. Acad. Sci. 63: 1-47. 1928.
21. Pochhammer, L. J. für Math., Crelle, 881: 324. 1876.
22. Quimby, S. L. Phys. Rev. 25: 558-573. 1925.
23. Rayleigh, Lord. Theory of sound, Vol. 1. Macmillan. 1894.
24. Swift, H. W. Phil. Mag. 2: 351-368. 1926.
25. Thornton, W. M. Phil. Mag. 38: 705-707. 1919.
26. Wyckoff, R. W. G. Structure of crystals, Chem. Cat. Co. 1924.

VELOCITY OF SOUND IN CYLINDRICAL RODS[1]

By Geo. S. Field[2]

Abstract

The experimental knowledge so far available of the velocity of longitudinal waves in cylindrical rods is reviewed, and it is shown that a close analogy most probably exists between waves in cylinders of liquid and in solid rods. The theory for rods due to Pochhammer is considered with reference to a specific case for which experimental velocities have been determined, and it is shown that the agreement at low frequencies is good. At higher frequencies, however, theory and experiment differ widely.

Introduction

In view of the results recently obtained (5), both experimentally and theoretically, in connection with the propagation of sound through liquids contained in cylindrical tubes, it was decided to investigate the possibility of similar results being obtained with solid cylindrical rods. It is to be expected that the introduction of an extra constant (Poisson's ratio) will modify the results, but we may look for the phenomenon of selective absorption at and near the frequencies of radial resonance for rods made of isotropic materials; and even with anisotropic materials something of the kind will most probably occur.

As a matter of fact, experiments which have already been performed have indicated that in the frequency ranges studied the velocity-frequency relation for solid rods is analogous to that for cylinders of liquid. In a paper by Boyle and Sproule (4) it is shown that at low frequencies, where $\left(\frac{rk}{l}\right)^2$ is a small fraction (r is the radius of the rod, l the length and k the mode of vibration), the velocity is not very different from $c_0 = \sqrt{\frac{E}{\rho}}$, where E is Young's modulus of elasticity and ρ is the density. The correction suggested by Rayleigh,(7, pp. 251-3) applies at slightly higher frequencies, but, for example, in duralumin when $\left(\frac{rk}{l}\right)^2 > 0.3$ the velocity decreases so rapidly with frequency that this correction is no longer applicable. The rapid drop in the velocity-frequency curve near a certain frequency, characteristic of the material and dimensions of the rod, is the same phenomenon that was observed for liquids in the region of anomalous dispersion. There then follows a range of frequencies for which no experiments have been conducted, but at very high frequencies, where $\left(\frac{rk}{l}\right)^2$ is very great, the researches of R. W. Boyle and coworkers (2, 3) have shown that the velocity is very high, approximately what would be given by taking the velocity equal to $\sqrt{\frac{K}{\rho}}$, where K is what Barton (1, pp. 125, 190) calls the "elongational

[1] *Manuscript received October 30, 1931.*
Contribution from the National Research Laboratories, Ottawa.
[2] *Junior Research Physicist, National Research Laboratories.*

elasticity", and is to be used for elongational waves in an infinite medium. This value of velocity corresponds to the value found for cylinders of liquid when the wave-length was very small compared with the radius, *i.e.*, when the liquid behaved as if it were an infinite medium.

Experiments are now under way in this laboratory to investigate the frequencies above the range where the velocity is very low, and it is hoped soon to demonstrate the complete phenomenon from very low frequencies to very high, corresponding to values of $\left(\frac{rk}{l}\right)^2$ from zero to infinity. In the meantime, existing theory has been closely examined to see if it will explain what is already known and perhaps indicate what is to be expected in the range where experiments are now being conducted.

A short time ago Ruedy (8) published an analysis of the theory due to Pochhammer (6) and showed that the velocity of a longitudinal wave in a solid rod would decrease steadily with increasing frequency. It was suggested that anomalous dispersion of sound waves would probably occur at the resonant frequencies of the radial vibrations, but no mention was made of the effect this phenomenon would have on the velocity.

In the following paper Pochhammer's equations are put into a form similar to those recently obtained for the velocity of sound in liquids contained in tubes. The equations are then applied to the case of a duralumin rod, for which some experimental data are available, and conclusions are drawn as to the probable accuracy of the theory.

Velocity Equation

The theory gives the following expression for the propagation of waves along a rod,

$$\left\{2\gamma^2 - \frac{p^2\rho}{\mu}\right\} J_1(k'a)\left\{J_1(h'a)\frac{2\mu h'}{a} - J_0(h'a)\left[\frac{p^2\rho\lambda}{\lambda+2\mu} + 2\mu h'^2\right]\right\}$$

$$= -4\gamma^2 h'\mu J_1(h'a)\left\{-\left(\frac{1}{a}\right)J_1(k'a) + k'J_0(k'a)\right\}, \tag{1}$$

where $\gamma^2 = \frac{p^2}{c^2}$, $p = 2\pi$ times the frequency (n), c = phase velocity of the wave, ρ = density of the material, a = radius of the rod, $\mu = \frac{E}{2(1+\sigma)}$, $\lambda = \frac{E\sigma}{(1+\sigma)(1-2\sigma)}$, E = Young's modulus, σ = Poisson's ratio, $h'^2 = \frac{p^2\rho}{\lambda+2\mu} - \gamma^2$, $k'^2 = \frac{p^2\rho}{\mu} - \gamma^2$, and J_0 and J_1 are Bessel's functions of the first kind of order zero and one.

If we substitute $\gamma^2 = \frac{p^2\rho}{\lambda+2\mu} - h'^2$ and write $h'a = x$ and $k'a = y$, Equation 1 may be rearranged to give,

$$x\frac{J_1(x)}{J_0(x)} = \frac{\left\{2x^2 + \frac{a^2p^2\rho\lambda}{\mu\lambda+2\mu^2}\right\}^2}{\frac{2a^2p^2\rho}{\mu} + 4\left\{x^2 - \frac{a^2p^2\rho}{\lambda+2\mu}\right\}y\frac{J_0(y)}{J_1(y)}} \tag{2}$$

Since $h'^2 - k'^2 = p^2\rho\left\{\frac{1}{\lambda+2\mu} - \frac{1}{\mu}\right\}$, we have an equation relating x and y

as follows,

$$y^2 = x^2 + \frac{a^2 p^2 \rho(\lambda+\mu)}{\mu\lambda + 2\mu^2} . \tag{3}$$

Now $$\frac{p^2}{c^2} = \gamma^2 = \frac{p^2\rho}{\lambda+2\mu} - h'^2 = \frac{p^2\rho}{\lambda+2\mu} - \frac{x^2}{a^2} . \tag{4}$$

And, $$\frac{\lambda+2\mu}{\rho} = \frac{E}{\rho}\left\{\frac{\sigma}{(1+\sigma)(1-2\sigma)} + \frac{1}{1+\sigma}\right\} = \frac{E}{\rho}\left\{\frac{1-\sigma}{(1+\sigma)(1-2\sigma)}\right\} . \tag{5}$$

Hence, if we put $c_0^2 = \dfrac{E}{\rho}$, we have,

$$\frac{p^2}{c^2} = \frac{p^2}{c_0^2}\left\{\frac{(1+\sigma)(1-2\sigma)}{1-\sigma}\right\} - \frac{x^2}{a^2} . \tag{6}$$

Rearranging, and writing $2\pi n$ for p, we obtain,

$$c^2 = \frac{c_0^2 n^2}{\dfrac{(1+\sigma)(1-2\sigma)}{1-\sigma} n^2 - \dfrac{x^2 c_0^2}{4\pi^2 a^2}} . \tag{7}$$

From Equation 2 it is possible to obtain x, and from Equation 7 the phase velocity, c, may then be determined.

It will be seen from the definition of h' and k' that these two quantities may be real or imaginary, depending upon the value of γ, which in turn is dependent upon phase velocity.

Equations Applied to a Specific Case

Consider a duralumin rod, with constants as follows: $a = 2.55$, $c_0 = 5.2 \times 10^5$, $\sigma = 0.36$, $\rho = 2.7$, $E = 7.3 \times 10^{11}$.

Now, $$h'^2 = p^2\left\{\frac{\rho}{\lambda+2\mu} - \frac{1}{c^2}\right\} . \tag{8}$$

And $$k'^2 = p^2\left\{\frac{\rho}{\mu} - \frac{1}{c^2}\right\} . \tag{9}$$

Substituting the numerical values, we have,

$$h'^2 = p^2\left(\frac{1}{(1.3c_0)^2} - \frac{1}{c^2}\right) \tag{10}$$

$$k'^2 = p^2\left(\frac{1}{(0.61c_0)^2} - \frac{1}{c^2}\right) . \tag{11}$$

From these equations we see that, since $h' = \dfrac{x}{a}$ and $k' = \dfrac{y}{a}$, we have three possibilities:

(a) if $c < 0.61\ c_0$, then x imaginary and y imaginary,

(b) $1.3c_0 > c > 0.61c_0$, then x imaginary and y real,

(c) $c > 1.3\ c_0$, then x real and y real.

For low frequencies we know experimentally that c approximately equals c_0. In any case it is in the range given by (b). Hence to begin with we use x imaginary and y real.

If the numerical values are then substituted in Equation 2, this equation becomes,

$$x' \frac{I_1(x')}{I_0(x')} = \frac{\left\{x'^2 - 7.3n^2\right\}^2}{-13n^2 + (x'^2 + 5.7n^2)y\dfrac{J_0(y)}{J_1(y)}} \tag{12}$$

where $ix' = x$, $J_0(ix') = I_0(x')$, $J_1(ix') = iI_1(x')$, and the frequency, n, is expressed in units of 10^5 cycles per sec.

From Equation 12 values of x' were determined for different frequencies as given in Table I.

TABLE I

SOLUTIONS OF EQUATION 12 FOR $1.3c_0 > c > 0.61c$

n in units of 10^5	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
x'	0.20	0.40	0.66	0.95	1.33	2.0	2.76	3.46

At frequencies of 90000 cycles/sec. and higher, for the rod under consideration, it is found that for a solution of x it is necessary to use Equation 12 with both x and y imaginary, corresponding to $c < 0.61\ c_0$. In the equation this means using $y' \frac{I_0(y')}{I_1(y')}$ instead of $y \frac{J_0(y)}{J_1(y)}$, where $y = iy'$; otherwise Equation 12 is unaltered.

Values of x' for the higher frequencies were then determined as in Table II.

TABLE II

SOLUTIONS OF EQUATION 12 FOR $c < 0.61c_0$

n in units of 10^5	0.9	1 0	1.2	1.4	2.0
x'	4.08	4.70	5.77	6.82	9.84

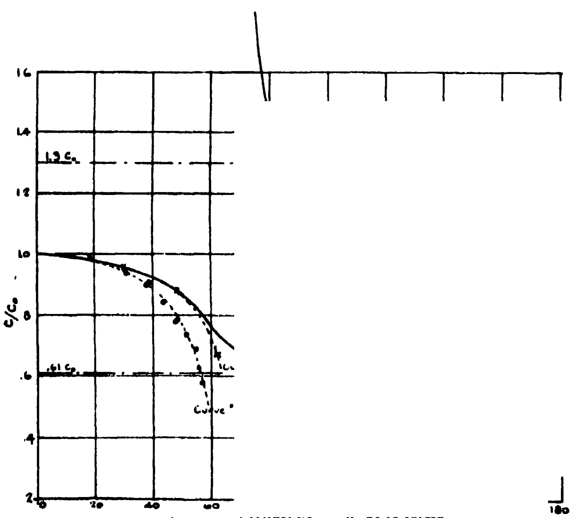


FIG. 1. *Velocity of sound in a cylindrical duralumin rod, showing theoretical and experimental curves.*

By substituting for x in Equation 7, and remembering that $(-x^2) = -(ix')^2 = +x'^2$, the velocity corresponding to each x' was determined. A curve of velocity against frequency was then plotted as in Fig. 1 (Curve No. 1).

If anomalous dispersion were occurring, we should expect a higher velocity than c_0 to exist for frequencies above the absorption frequency. Accordingly, solutions for Equation 2 were sought in the range $c > 1.3c_0$; that is, with x and y real. Table III shows the results.

TABLE III

SOLUTIONS OF EQUATION 12 FOR $c > 1.3\ c_0$

n in units of 10^5	0.75	0.80	0.83
x	1.23	.78	.45

For frequencies of 85000 and over, in order to obtain a solution it was necessary to use Equation 2 for the range $1.3c_0 > c > 0.61\ c_0$; that is, x imaginary and y real. Values of x' so determined are given in Table IV.

TABLE IV

SOLUTIONS OF EQUATION 12 FOR $1.3c_0 > c > 0.61c_0$

n in units of 10^5	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.8	3.0	6.0
x'	0.74	1.27	1.72	2.2	2.79	3.46	4.18	6.22	12.5	26.7

By using Equation 7 and the values of x' and x from Tables III and IV, velocities were determined for the corresponding frequencies, and were plotted in Fig. 1 (Curve No. 2).

TABLE V

Resonant frequency in kilocycles/sec.	Supposed harmonic	Velocity of wave in cm./sec.
22.7	5	4.88×10^5
30.8	7	4.73
37.6	9	4.50
38.3	9 (?)	4.58
43.3	11	4.23
47.2	13	3.91
48.2	13 (?)	3.98
51.6	15	3.70
54.8	17	3.46
55.6	19	3.17
56.9	21	2.89
57.5*	—	—
58.1*	—	—
59.1	?	?
59.7	?	?
61.0	?	?
62.0	?	?
65.3	?	?
Etc.	?	?

**Continuous resonance—probably here velocity so low that harmonic frequencies are too close to be separable.*

On the same figure (Curve No. 3) have been plotted for comparison a few velocities obtained *experimentally* by Boyle and Sproule (4) for the duralumin rod considered above. Another experimental curve (No. 4) is shown which was obtained for a cylinder of somewhat larger radius (3.38 cm.), as this curve shows somewhat better the very rapid decrease in velocity which occurs near a definite frequency for each cylinder. The experimental velocities determined by method (5) of Boyle and Sproule (4) for a number of frequencies, are given for this case in Table V, and it is there shown that a discontinuity exists at a frequency in this case of about 57 kilocycles/sec.

Conclusions

From what is experimentally known about the velocity of sound in rods, any theory which purports to explain the facts should give certain velocity-frequency relations as follows:

(1) Starting with a value c_0 at very low frequencies, *i.e.*, $\left(\frac{rk}{l}\right)^2$ a small

fraction, the velocity should decrease steadily with frequency, until,

(2) At a certain frequency the velocity is decreasing so rapidly that it is impossible to obtain resonance points with a rod, and an apparent discontinuity in the velocity-frequency curve exists.

(3) At very high frequencies the velocity in the rod is comparatively high, being of the order of what would be given by using the elongational modulus of elasticity instead of Young's modulus in the equation $c = \frac{E}{\rho}$.

The theory due to Pochhammer, as has been demonstrated in the preceding analysis, indicates a decreasing velocity with increasing frequency, and the agreement at the lower frequencies with experimental determinations is very good. However, there is no discontinuity in the theoretical curve, and the velocities at very high frequencies are not greater than c_0, but much less. It is to be noted, therefore, that the theory which has been discussed accounts for only one, *i.e.*, the first, of the three velocity-frequency relations which are already experimentally known.

Added Note

That the high velocities at high frequencies mentioned in experimental velocity-frequency relation No. 3 are not obtainable from Pochhammer's equations may be shown in the following way:

If $x = 0$ in Equation 7, the velocity is the same as is given by Barton (1, pp. 125,190) for an infinite medium, *i.e.*, equivalent to $c = \sqrt{\frac{\text{elongational modulus}}{\text{density}}}$. This is the velocity that experimentally has been found to occur at high frequencies, where $\left(\frac{rk}{l}\right)^2$ is very large.

Substituting $x = 0$ in Equation 12, we get,

$$0 = \frac{53.5n^4}{-13n^2 + 5.7n^2\left(4.55n\,\frac{J_0(4.55n)}{J_1(4.55n)}\right)}\,.$$

But this equation, although satisfied at intervals, *i.e.*, when $\frac{J_0(4.55n)}{J_1(4.55n)} = \infty$, is not satisfied for a *range* of high frequencies (in fact, if correct it should be satisfied for *all very high* frequencies, as the rod should then behave as an infinite medium, and has been shown so to do (2, 3).

In conclusion, therefore, the theory accounts for the right velocity when $n \longrightarrow 0$, but does not do so when $n \longrightarrow \infty$.

References

1. Barton, E. H. A text book on sound. Macmillan, 1908.
2. Boyle, R. W. and Froman, D. K. Can. J. Research, 1: 405-424. 1929.
3. Boyle, R. W. and Sproule, D. O. Can. J. Research, 2: 3-12. 1930.
4. Boyle, R. W. and Sproule, D. O. Can. J. Research, 5: 601-618. 1931.
5. Field, G. S. Can. J. Research, 5: 132-148. 1931; Nature, 128: 117. 1931.
6. Pochhammer, L. J. Math. (Crelle), 81: 324-336. 1876. (Summarized by A. E. H. Love in a treatise on the mathematical theory of electricity. Camb. Univ. pr. 1927.
7. Rayleigh, Lord. Theory of sound, 2d ed. v. 1. Macmillan. 1894.
8. Ruedy, R. Can. J. Research, 5: 149-155. 1931.

THE IONIZATION OF THE ATMOSPHERE MEASURED FROM FLYING AIRCRAFT[1]

By D. C. Rose[2]

Abstract

The Gerdian type of atmospheric ionization measuring apparatus was attached to a cabin aeroplane so that the state of ionization of the atmosphere could be studied. The limitations of the apparatus for aeroplane use are discussed. Measurements were taken from ground level to heights of 15000 ft. The results are plotted in number of ions per cc. (separate curves for positive and negative) at different altitudes.

The results indicate that at the cloud level there is an abnormal excess of small positive ions and a minimum in the excess of positive ions over negative ions from 4000-6000 ft. higher. This does not include large ions such as charged water drops or dust particles. The observations were taken in regions free from clouds, the cloud level being determined by observation on clouds in the sky, and by relative humidity measurements taken at the same time.

A great deal of work has been done on the conductivity or state of ionization of the atmosphere at ground level*. Diurnal and yearly variations have been found both on land and at sea for which no adequate explanation has been brought forward. The present paper deals with an attempt to study the variation in the ion content of the air at different altitudes. Little attention has been paid to the absolute value of the number of ions per cc. in the air but the relative values at different altitudes have been studied with a view to correlating variations in the ionic content of the atmosphere with weather conditions. This work was undertaken in connection with some work on the elimination of static charging of the films in the cameras used for aerial photography, for which it was found advisable to measure relative humidity and other atmospheric conditions in the slip stream of flying aircraft. The results of the relative humidity experiments are published elsewhere (5).

Method of Measurement

The instrument used for measuring the state of ionization in the air was the Gerdien conductivity apparatus. A sketch of the apparatus as used for these experiments is shown in Fig. 1. The ion tube was of brass, 5.43 cm. in diameter and about 14 in. long. The collecting electrode was a copper rod the effective length of which was taken as 29.2 cm., the diameter being 0.63 cm. The collecting electrode was connected to a Wulf type of fibre

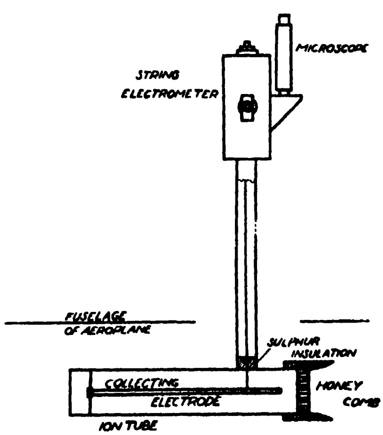


Fig. 1. *Apparatus used for measuring the conductivity of the air.*

**Publications of the Department of Terrestrial Magnetism, Carnegie Inst., Washington.*

[1] *Manuscript received November 16, 1931.*
Contribution from the National Research Laboratories, Ottawa, Canada.

[2] *Assistant Research Physicist, National Research Laboratories, Ottawa.*

electrometer by a fine wire tightly stretched through the centre of a side tube brazed to the ion tube as shown in the figure. The function of the honeycomb and the position of the apparatus in the aeroplane will be described in a latter section.

Theory and Method of Reduction of Observations

The theory of this type of ion measuring apparatus has been considered carefully by Swan (8). He shows that for the general case the rate of loss of an insulated charged body in a stream of air is given by the equation:

$$-\frac{dQ}{dt}=4\pi QneM, \tag{1}$$

where Q is the charge on the central collector, n the number of ions per cc. in the air (assumed sufficiently small that it has no effect on the electric field), e the charge on the ions and M the mobility of the ions. To put the equation in a working form Swan has shown very simply that when the insulated rod and part exposed to the air has a measured capacity C, and the total insulated system (rod, electrometer element and connections) C_1, the equation may be reduced to the form:

$$-C_1\frac{dV}{dt}=4\pi CVneM \tag{2}$$

where V is the potential of the insulated system.

Actually the capacity of the insulated system was not measured, but treating it as a cylindrical condenser and neglecting corrections for the end of the rod and for the parts connecting it to the electrometer which are exposed to the air, the equation may be written:

$$-C_1\frac{dV}{dt}=\frac{2\pi neMLV}{\log\frac{b}{a}}, \tag{3}$$

where L is the length of the collecting rod and b and a the radii of the ion tube and collecting rod respectively. This equation can be integrated easily but in the present experiment it was used in the differential form:

$$-ne=\frac{C_1\log\frac{b}{a}}{2\pi MLV}\frac{dV}{dt}. \tag{4}$$

As Swan has pointed out, this formula as used originally by Gerdien is only approximately true and unless the length of the tube and electrode is many times its diameter the error in using the above formula may be large. In the present case the error would be much smaller than in the case described by Swan (error of 39%) as the tube was much longer in comparison to its diameter. Also these results are necessarily of a preliminary nature as only four flights were taken and the adaption of these ionization measuring instruments to aeroplane use was by no means simple. Other sources of error will be discussed below.

If we call M_0 the mobility of the ions concerned at a temperature t_0 and pressure p_0 the value of M in Equation 4 is:

$$M=M_0\frac{P_0}{P}\frac{t}{t_0}.$$

Further, putting S equal to the sensitivity of the electrometer in electrostatic units per division Equation 4 becomes:

$$ne = \frac{C_1 \log \frac{b}{a} P t_0 S}{2\pi M_0 P_0 t L V} \frac{dx}{dt}, \tag{5}$$

where x is the scale reading of fibre in the electrometer. The value of C_1 was found by measurement to be 37.4×10^{-12} farads. It was measured by the method of mixtures using a calibrated Leybolds cylindrical condenser as a standard and the electrometer itself as a potential measuring device. P was measured throughout the flight by a Tycos aneroid barometer. The temperature was taken from the dry bulb thermometer used in the humidity measuring device carried alongside the ion tube.

The procedure followed in taking readings and working out the results was as follows: During the flights, with the exception of the first, the plane was flown level at the altitude being studied for a period long enough to take four or five good readings of the position of the electrometer fibres on the microscope scale. Then the plane was flown to the next level, the insulated system recharged and another set of readings taken. In plotting the results the actual readings of the electrometer were plotted for each altitude. A tangent was drawn to a smooth curve through the points (usually nearly a straight line) and the slope of the tangent was measured. This gives a value of $\frac{dx}{dt}$ at the same value of x for each altitude or at the same value of the potential. Reducing the results in this manner the potential at which the observation is taken may be considered as constant during the particular flight in question, and the relative value of the quantity ne can be calculated from the simpler relation:

$$ne = K \frac{P t_0}{t} \frac{dx}{dt}, \tag{6}$$

K being a constant which changes for each flight and for different varieties of ions. The quantity plotted in Fig. 2 is the value of $\frac{P t_0}{t} \frac{dx}{dt} \times 10^{-3}$, P being in millibars and $\frac{dx}{dt}$ in divisions per minute: t_0 was not included in the value of K for the sake of simplicity in calculation because when the temperature t did not vary by more than about 10° C. the temperature correction was neglected as it would be smaller than other possible errors.

Mobility

To obtain a measure of the number of ions per cc. in the air it now remains to evaluate K. The only quantity which cannot be determined easily is the value of M_0, the mobility at N.T.P. A review of recent literature on mobilities of ions in air shows that there are still conflicting results. The mobility as measured in the laboratory varies not only with pressure and temperature but with humidity, purification of the air, potential, etc. Also, without doubt there is more than one type of ion of the same sign involved.

The difficulty might be overcome by plotting the results in positive and

negative conductivity, as is more often done, rather than number of positive or negative ions per cc. In the present case, as the variation in space charge with altitude was being studied, it seemed more appropriate to assume the most likely mean mobility and evaluate K from it. From the work of Zeleny (13), Hamshere (1) and Nolan and Nevin (4), it seems reasonable to assume a mean mobility of 1.8 for negative ions and 1.2 for positive ions and the results are computed on that basis. It should also be pointed out that heavy ions found in the atmosphere, such as the dust particles, are also omitted in this discussion as the apparatus would collect only a negligible number of them.

Aerodynamics of the Ion Tube

Before proceeding with the results there are some limitations to the application of this Gerdien type of conductivity or space charge apparatus. First assuming a streamline flow in the tube there is a definite radius r_0 of a cylinder of the air passing through the tube from which all the ions are collected. The value of r_0 must be less than b, the radius of the ion tube, otherwise the tube becomes saturated and the number of ions per cc. then depends on the velocity of the air through the tube bearing a relation totally different from Equation 5. As the velocity of air in the tube was not measured it is not advisable to allow this to take place. It may be shown that the value of r_0 is given by the equation:

$$r_0^2 = \frac{2M\,VL}{v \log \frac{b}{a}}, \tag{7}$$

where v is the velocity of the air stream. Swan uses $(r_0^2 - a^2)$ in place of r_0^2 on the left side of the equation and assumes that all the ions in the cylinder of radius a strike the end of the collecting rod. If the air flow around the rod is streamline, they probably do not because the air within the cylinder of radius a would be spread and only a few of the ions in it would strike the front end of the rod. A more correct value for the left hand side of Equation 7 is $(F^2 r_0^2 - a^2)$, where F is a function slightly greater than unity. This correction, however, can be omitted as when r_0 is of the order of b, a^2 can be neglected. A rough calculation of the limit of r_0 is all that is required.

The aeroplane was normally flown at a speed of between 70 and 80 miles per hour. The ion tube was in the slip stream which would have a considerably higher velocity than that of the plane, but the speed of the air through the tube would not be as high as that of the stream in which it was placed. Hence, taking the speed to be about 70 miles per hour, under average conditions, the value of r_0 from Equation 7 was about 2 cm. which was quite satisfactory. At the highest altitudes, where the mobility of negative ions is increased due to the low pressure, the value of r_0 becomes dangerously near the upper limit ($b = 2.7$ cm.) for which Equation 5 is applicable. Any error in this calculation due to the assumption of too low a mobility would mean that the results for the number of negative ions per cc. at high altitudes are too low.

Swan has shown that it is not necessary for the air in the ion tube to have a constant velocity over its cross section. However, when the air in the tube

becomes very turbulent, Equation 5 can no longer hold. The velocity in the tube would vary enormously with time, cross section, and length and the conditions that $r_0 < b$ would be meaningless. The condition for streamline flow in such a tube is that Reynolds' Number, R, should be less than about 1500. Reynolds' number is given by

$$R = \frac{vd}{u},$$

where v = velocity in feet per sec., d = diameter of opening in feet and u = the kinematic coefficient of viscosity, usually taken as 0.000159. At 70 miles per hour R becomes about 121,000 for this tube*. Hence the air flow in the tube was very turbulent.

In order to remove this turbulence and make the airflow in the tube such that the state of ionization could be measured by the relations discussed above, a honeycombed opening was designed to fit the intake end of the ion tube as shown in Fig. 1. It consisted of ⅛-in. square openings placed in a streamline cone the inside diameter of which was the same as the inside diameter of the ion tube. No doubt the honeycomb had some effect on the value of the results. Scholz (6) has done some experiments using this type of ion tube. Although his experimental conditions were somewhat different from those described here, he showed that the passage of the air through other tubes before it reaches the collecting electrode has some effects on the results. However, the error would be in the absolute value, not in the relative values at different altitudes. Probably the honeycomb does not remove all turbulence as there may be some in the smaller openings but the resulting flow through the tube would be laminar and hence Equation 5 should apply.

The Electrometer

The electrometer was of the fibre type. For Flights 1, 2 and 3 double fibres were used, the separation of the fibres being measured on a scale in the eyepiece of a microscope. The tension on the fibres was maintained by a quartz bow. The fibres were connected to the insulated system, the insulation throughout being of sulphur. The knife edges together with the ion tube and shields were earthed to the frame of the aeroplane. Sensitivities of from 6 to 10 volts per division at potentials of about 200 volts were easily obtainable and were found very satisfactory. Much greater sensitivities could be obtained at lower voltages by applying potentials to the knife edges but this would involve the carrying of a large number of batteries. The insulated system was charged by a 90-volt battery which charged a variable condenser. The battery was then disconnected, the capacity of the condenser was reduced until the insulated system reached the required potential (potentials of 400 or 500 volts could be obtained easily in cold weather by this means but the instrument was usually operated between 150 and 250 volts), then the condenser was disconnected from the insulated system.

On the fourth flight, a different type of fibre suspension was used having

Dr. J. J. Green of the Aeronautical Section of the National Research Council kindly helped the author in this phase of the problem.

only one fibre which, with one knife edge, acted as the insulated element of the electrometer. This was found more satisfactory to construct and calibrate but gave more trouble due to vibration. Although at all times the vibration of the aeroplane made the readings very difficult, usually with the double fibre suspension good readings could be obtained without disturbing the normal course of flight. In the case of the single fibre suspension the vibration was much more noticeable and in some cases readings could be taken only by arranging for the pilot to throttle down the engine and glide for the few seconds required. The ion tube, with the other apparatus, was suspended through the camera hole in the floor of the aeroplane. It was supported by rubber tubing with the intention of reducing the vibration to a minimum. The supports, however, were not entirely satisfactory as the above discussion indicates.

The electrometer was calibrated in the laboratory before and after each flight, but the accuracy of calibration was never very great because usually it was found to have changed slightly each time due to the rough treatment necessary in installing the instrument in the plane. The same fibre was used in the first two flights but it did not survive the dismantling after the second. In Flights 3 and 4 the fibre was again broken before it could be recalibrated after the flight. This places some doubt on the absolute value of the number of ions per cc. but by comparing different calibrations it is estimated that the error should not be greater than 10 or 15%. The relative values of course are much more accurate as the value of $\frac{dx}{dt}$ was determined at the same deflection for each point on the curves, and there is every reason to believe that the calibration remained reasonably constant during flight.

Charge on Aircraft

Another possible influence on the results of these observations should be mentioned. Wigand (10) has shown that aeroplanes will acquire a positive charge while in flight due to the fact that the exhaust gases from the engine are negative. This would attract negative ions to the aeroplane and give a result which might show the right variations with altitude but wrong absolute values. However, it is unlikely that this charge has any effect because of the velocity with which the plane moves through the air. The ion tube was about three metres from the front of the plane and about 15 cm. from the fuselage. At a velocity of 25 metres per sec. through the air, the potentials around the fuselage would have to be very high indeed to create sufficient potential to move the ions 15 cm. during the time (about $\frac{1}{8}$ sec.) the air stream flows from the front of the plane to the position of the ion tube.

The action of the propeller on the air may also have had some effect on the results. McDiarmid (3) measured the charge on an insulated tube when dry dust-free air flows through it rapidly. Her results showed a charging of the tube and a corresponding charge of opposite sign in the air. The direction of the sign depended on the material of which the tube was made. In the case of aluminium the charge was usually negative, leaving the air positive. Hence in these experiments the propeller (aluminium alloy) moving through the air

might have some effect on the results. The magnitude of this effect would be difficult to estimate though it would not be expected to be great. It should have been eliminated by placing the ion tube out of the slip stream on a wing strut or some such part of the plane. It would be very difficult to do this because of the necessity of having the electrometer in the cabin. It was therefore not attempted during these flights.

Results

The results of the observations taken during flight have been reduced to the curves shown in Fig. 2 by the methods outlined in the previous sections. The abscissas represent the altitude in thousands of feet. This was read in two ways, on the aircraft altimeter and on the Tycos aneroid barometer carried beside the ion tube. A reduction of the barometer readings to feet agreed within 100 or 200 ft. with the altimeter readings in Flights 3 and 4 but showed considerable disagreement in Flights 1 and 2. The barometer readings, however, were assumed to be the more accurate. In the case of the ordinates, the figures on the left of the vertical axis represent the value of $\frac{Pt_0}{t}\frac{dx}{dt} \times 10^{-3}$ as indicated in Equation 6. It is plotted this way because of the fact that the absolute values of n cannot be known nearly as accurately as the relative values. The higher figures inside the axis represent the value of n in number of ions per cc., assuming that each ion has a charge equivalent to one electron and values for mobilities as discussed above. The curves are drawn by joining the points by straight lines.

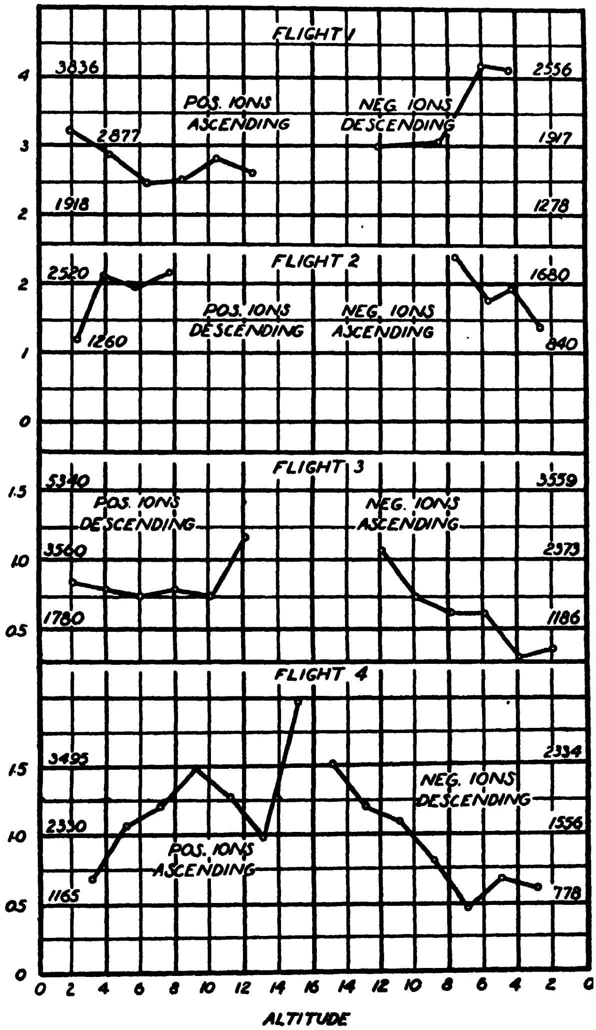


FIG. 2. *The state of ionization of the air at different altitudes. The ordinates are expressed in arbitrary units and in number of ions per cc.*

The curves representing the results of Flights 2, 3 and 4 show considerable similarity, particularly Flights 3 and 4, while Flight 1 is totally different. The reason for this, as mentioned in a previous section, is that in Flight 1 the ion tube was not equipped with the honeycomb. For this reason it will not be included in further discussion. As would be expected, the number of positive ions per cc. is greater than the number of negative. As one ascends, the number of ions of both signs shows a general tendency to rise, but the number of negatives rises more rapidly at first than the number of positives. This is more readily seen if one plots the difference between the number of positive and number of negative ions per cc. at different altitudes. This has been done in Fig. 3.

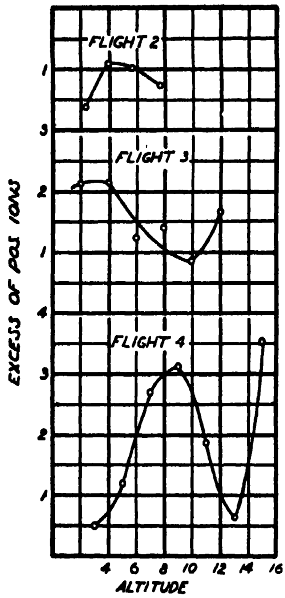


FIG. 3. *Excess of positive ions over negative. The ordinates represent thousands of ions per cc.*

Suppose we examine first the results of Flight 4. The excess of positive ions increased until an altitude of 9000 ft. was reached, then decreased rapidly to a minimum at 13000 ft. The shape of the curve for Flight 3 shows the same maximum and minimum as in the case of Flight 4 at altitudes of about 3000 and 9000 ft. respectively. In the case of Flight 2, an insufficient number of points was taken though the curve does indicate a maximum at about 4500 ft. A comparison of these results with weather conditions and the relative humidity (5) curves taken during the same flights is interesting.

Flight 2 was taken during a day (March 2, 1931) on which there was a thin layer of clouds about 3000 ft. high, from which occasional flurries of snow were falling. During the flight, clouds were avoided as much as possible. The sky was not totally overcast so clear places could always be found. The relative humidity readings show a maximum at about 4000 ft. (The aircraft altimeter read 5000 ft. but the barometer recorded a pressure which indicated about 4000 ft. Negative ions were studied during ascent and positive ions during descent.

In the case of Flight 3 (April 18, 1931) the record of the weather was not kept so accurately; but the sky was either clear or very few cumulus clouds were present. The relative humidity curves show a maximum at 4000 ft. or about the same altitude as the maximum excess of positive ions.

In Flight 4 the maximum in the curve in Fig. 3 again occurs at the same altitude as the relative humidity peak. The weather during this flight (June 17, 1931) was fine but there were numerous cumulus clouds at altitudes between 7000 and 9000 ft. During the flight these clouds were avoided as much as possible and in no case was the plane flown through or near them.

Although no great accuracy can be expected from these results, as has been indicated in the descriptions of the method of measurement, these three flights

were taken under sufficiently different conditions and the results are sufficiently consistent to show a definite indication that there is some change in the ionic content of the air about the cloud level. The change manifests itself by a decrease in the excess of the number of positive over the number of negative ions and the maximum of that excess appears just at the cloud level. A minimum in that excess then appears 4000 to 6000 ft. higher.

Conclusions

To compare the results of these experiments with others is difficult because as far as the author is aware no similar observations have been taken under similar circumstances. The great majority of ionization measurements have been made on the ground. Wigand in 1914 (11) and in 1921 (9) measured the conductivity of the air with a similar apparatus during flights in a manned balloon and found that in general the conductivity increased with altitude, the positive conductivity remaining higher than the negative. This does not necessarily mean that the number of ions per cc. increases with altitude as the conductivity follows the relation $\lambda = neM$ and the mobility M increases with reduced pressure. His results show that at levels where there is mist the value of $\frac{\lambda+}{\lambda-}$, the ratio of positive to negative conductivities, usually assumes a lower value than in clear places, often going below unity. These experiments are in agreement with the results of the present investigation in that the number of positives were apparently decreased relative to the number of negatives in or just above clouds.

Lugeon (2) measured the number of ions per cc. in the air at altitudes of 2450 and 4358 metres (8040 and 14300 ft.) and found that in daytime the total number of ions was less at the higher level than at the lower. His observations were taken on mountains and therefore would be expected to differ somewhat from those in free air due to the influence of the mountain. He records that as a cloud came up the mountain past the station, a greater excess of positive ions was noted above the cloud and relatively more negatives were found in the cloud. In the present experiments no visible cloud existed where measurements were taken but there is an indication of a cloud level in the humidity curves. It will appear later that it is difficult to compare his results with those obtained by the author because it is not clear whether or not Lugeon's results included both heavy and light ions.

Wigand (10) made some more recent measurements of potential gradient from which the resulting space charge can be deduced by using Poisson's equation. His measurements were taken during flights in an airship. He found that normally the potential gradient on the ground is positive and decreases in such a way that the space charge diminishes with height. On one of his ascents he found an inversion in the potential gradient slope which indicates an excess of negative space charge between 700 and 800 metres altitude (2296 and 2625 ft.). At higher altitudes the resulting space charge became small. This inversion in the space charge or a decrease in the excess of positive ions in or near cloud levels was noticed in early experiments by Elster and

Geitel, and Linke, which are discussed by Wigand. They introduced a theory for this inversion based on the normal upward movement of negative ions and downward movement of positive ions. These ions when coming into a region of mist or haze become attached to heavy particles and their normal mobility becomes greatly reduced. Hence at the top of a layer of mist or haze there would be an accumulation of positive ions and at the bottom an accumulation of negative ions. Wigand on one flight noted the accumulation of negative ions at the bottom, but found no corresponding excess of positive at the top. As ascents were not above 1300 metres, he may not have been high enough. It is obvious that this action could not go on for any length of time as the electric fields built up would tend to neutralize it.

In the present experiments the peak in the curves representing the excess of positive ions over negative may be more apparent than real because of the greatly lower mobility of heavy ions. The apparatus used would measure a negligible number of heavy ions, so if at the cloud level the negative ions were made ineffective by condensation, the results would indicate a greater excess of positives in a region where there might really be considerable negative space charge as well as positive. The minimum in the curves in Fig. 3 might be explained in an analogous manner. The increase in the excess of positive ions at the highest level is not as easily understood, though if condensation nuclei are to be used for the explanation, it might be due to the lack of water vapor.

Another explanation of the effect seems equally tenable. One assumes first that this stratification of the electricity in the air is never, under normal fine weather conditions, sufficient to reverse, to any great extent, the normal positive potential gradient. In expansion apparatus of the Wilson type it has been found that water condenses on negative ions with smaller expansions than on positive. As negative ions ascend in the atmosphere they reach temperatures and pressures at which they become condensation nuclei and so would not be included in the measurements taken in the present experiments. The result is the apparent excess of positive ions at the cloud level. Positive ions would condense higher up and so show a minimum as in the curves in Fig. 3. Whether the positive ions come from above or below does not matter as the amount of water vapor available for condensation is rapidly decreasing. In order to get sufficient data to prove or disprove these theories, potential gradient measurements would have to be taken at the same time as ionization measurement.

The cumulus clouds in the sky during Flight 4 were those which often develop into local thunderstorms or the cumulo-nimbus type. The work of Schonland (7) and Wormell (12) seems to indicate that the majority of thunderclouds are of positive polarity. That is, the centre of positive charge is above the centre of negative charge. Their conclusions were drawn from vertical current measurements on the ground during thunderstorms in England and South Africa. The present observations were taken in spaces free from visible clouds though in Flight 4 there were numerous cumulus clouds in the sky. The

process of the development of the thundercloud has never been satisfactorily explained. Assuming that the results of the present experiments indicate a layer of heavy negative ions at the cloud level and heavy positive ions higher up, in so far as the heavy ions or charged mist particles are concerned, there is a normal electrical stratification in the same direction as that found in thunderclouds. The magnitude of the charges must, of course, be negligible compared to those in a thunderstorm.

In view of the preliminary nature of these results the conclusions which have been drawn must be considered only as tentative.

A great many more observations should be made with potential gradient apparatus as well as ionization measuring equipment. The author hopes to be able to carry on this work with more elaborate equipment. With the experience gained in the present work, valuable results should be obtainable.

Acknowledgment

The author wishes to take this opportunity to thank the Aeronautics Branch of the Department of National Defence, particularly the officers who undertook the flying.

References

1. Hamshere, J. L. Proc. Roy. Soc. Lond. A, 127: 298-314. 1930.
2. Lugeon, J. Compt. rend. 191: 110-112. 1930.
3. McDiarmid, Miss A. W. Phil. Mag. 6: 1132-1140. 1928.
4. Nolan, J. J. and Nevin, T. E. Proc. Roy. Soc. Lond. A, 127: 155-174. 1930.
5. Rose, D. C. Can. J. Research, 5: 482-489. 1931.
6. Scholz, J. Physik. Z. 32: 130-139. 1931.
7. Schonland, B. F. J. Proc. Roy. Soc. Lond. A, 118: 233-251. 1928.
8. Swan, W F. G. Terrest. Magn. 19: 81-92. 1914.
9. Wigand, A. Ann. Physik, 66: 81-109. 1921.
10. Wigand, A. Ann. Physik, 85: 333-361. 1928.
11. Wigand, A. Terrest. Magn. 19: 93-101. 1914.
12. Wormell, T. W. Proc. Roy. Soc. Lond. A, 127: 567-590. 1930.
13. Zeleny, J. Phys. Rev. 36: 35-43. 1930.

STUDIES ON HOMOGENEOUS FIRST ORDER GAS REACTIONS

I. THE DECOMPOSITION OF ETHYLIDENE DIACETATE[1]

By C. C. Coffin[2]

Abstract

The decomposition represented by the general equation

$$\mathrm{R'CH}\begin{cases}\mathrm{OOCR''}\\ \mathrm{OOCR'''}\end{cases} \rightleftharpoons \mathrm{R'CHO} + \mathrm{O}\begin{cases}\mathrm{OCR''}\\ \mathrm{OCR'''}\end{cases}$$

has been found to take place according to the monomolecular law. In the case of the several homologous esters already investigated at pressures above 10 cm. of mercury the reaction is entirely homogeneous, is uninfluenced by the presence of inert gases and obeys the Arrhenius equation. This paper describes the experimental method and deals with the decomposition of ethylidene diacetate to acetaldehyde and acetic anhydride at temperatures of 220° to 268° C. and at initial pressures of 11 to 46 cm. of mercury. The heat of activation is 32900 cal./mol and the velocity constants (sec^{-1}) are given by the equation, $\ln k = 23.74 - \frac{32900}{RT}$. The theoretical significance of the data is discussed.

Introduction

The theoretical significance attached to monomolecular gas reactions has recently led to the discovery of a considerable number of examples of this type of chemical change. Thus, while four or five years ago the decomposition of nitrogen pentoxide was the only generally recognized instance of such a reaction, there are now at least 14 compounds which are known to decompose by the monomolecular mechanism under certain conditions of pressure and temperature (18). However, the fact that more than half of these substances fall into either one of two classes—the aliphatic ethers investigated by Hinshelwood and his students (3, 5, 6, 7, 8, 9, 10) and the aliphatic azo compounds studied by Ramsperger (14-18)—gives an idea of the rarity of these reactions and the desirability of finding other examples with which to test current theories of molecular activation and reaction mechanism.

In the course of a systematic investigation of equilibria and reaction velocities in acid anhydride-aldehyde-ester systems from points of view outlined elsewhere (1, 2) a new series of monomolecular reactions has been found, *viz.*, the decomposition represented by the general equation

$$\mathrm{R'CH}\begin{cases}\mathrm{OOCR''}\\ \mathrm{OOCR'''}\end{cases} \rightleftharpoons \mathrm{R'CHO} + \mathrm{O}\begin{cases}\mathrm{OCR''}\\ \mathrm{OCR'''}\end{cases}$$

where R$'$, R$''$ and R$'''$ as usual represent organic radicals. The several homologous esters of this type that have already been examined in the gaseous state at pressures of 10 to 40 cm. of mercury break down homogeneously according

[1] *Manuscript received September 15, 1931.*
Contribution from the Department of Chemistry, Dalhousie University, Halifax, Canada.

[2] *Assistant Professor of Chemistry, Dalhousie University.*

to the monomolecular law at a measurable velocity, under conditions where the equilibrium for practical purposes is shifted completely to the right. The present paper describes the experimental procedure and deals with the decomposition of ethylidene diacetate, $CH_3CH(OOCCH_3)_2$, which breaks down quantitatively to acetic anhydride and acetaldehyde. The velocity of the reaction has been determined at initial pressures of 11 to 46 cm. of mercury between the temperatures of 220 and 268° C. Rate measurements at lower pressures are being carried out at present in another apparatus as a test of Lindemann's hypothesis (13) regarding the activation mechanism of a monomolecular reaction. The decomposition of other esters of the series will be dealt with in subsequent papers.

Experimental

Apparatus and Technique

The low volatility of these esters (b.p. ethylidene diacetate = 172° C.) makes it difficult to prevent condensation, particularly at the higher pressures, in the manometer and other tubing connected with the reaction chamber, so that a considerable amount of preliminary experimentation was necessary before a satisfactory technique was developed and traces of liquid phase were completely eliminated. The method finally adopted is sufficiently different from

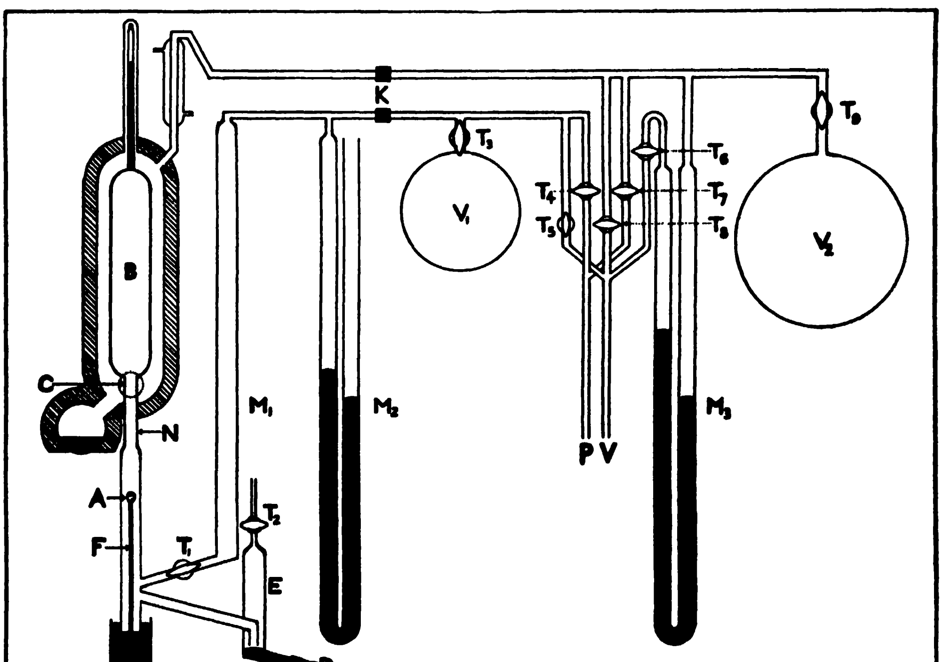


FIG. 1. *Diagram of apparatus.*

that usually employed in such work to warrant a rather detailed description. Its main disadvantage is that the reaction is carried out in the presence of mercury vapor saturated at the temperature of the reaction chamber. The

fact, however, that the logarithm of the velocity constant plotted against the reciprocal of the absolute temperature results in a straight line is evidence that for these high pressure runs at least mercury vapor is without influence on the reaction rate. This point is being studied more fully in connection with the above-mentioned low pressure decompositions.

The apparatus, which is shown in Fig. 1 (not drawn to scale), consists essentially of a reaction chamber, B (volume, 120 cc.), surrounded by the vapor of mercury boiling under a definite pressure, together with the manometers, connections to vacuum, etc., necessary for following the reaction by change of pressure at constant volume. All the apparatus to the left of the deKhotinsky seals, K, was built of Pyrex glass. The procedure in carrying out an experiment was as follows.

The mercury in the tube E was set at about the level indicated in the diagram by raising or lowering a mercury reservoir (not shown) attached to D by heavy rubber tubing which was then clamped. The rubber stopper bearing the glass tube F (closed at the bottom) was removed and a small glass bulb A almost filled at room temperature with a weighed amount of the ester was inverted in the open end of F. The rubber stopper was then replaced, surrounded by a mercury seal, and B was evacuated through P by means of two mercury condensation pumps backed by a Hyvac.

The glass jacket surrounding B was provided with a mercury boiler (gas heated), a water cooled condenser and a thermometer as shown in the diagram. All connections were glass-sealed and the whole was heavily lagged with asbestos. The pressure on the boiling mercury was adjusted to any desired value by taps T_7 and T_8 and read on the manometer M_3. V_2 represents a 12-litre stabilizing volume. The temperature of B was determined from the pressure registered on M_3, the vapor pressure data of the I.C.T. (Vol. III) being used. Throughout all the runs reported the temperature was constant to within 0.2° C. and was probably correct to at least 0.2° C. as the manometer readings were corrected to 0° C. and all the mercury used in boiler and manometers was carefully cleaned in nitric acid and distilled at least twice in a stream of air.

When B had been thoroughly evacuated and brought to a constant temperature, the clamp at D was opened and the mercury was allowed to rise slowly in M_1 and in N where it picked up the bulb A and floated it up into B. As the bulb and the mercury surface disappeared under the asbestos insulation, the tap T_1 was closed and the mercury rose rapidly in the left hand side until it reached the mark C, etched on the neck of the bulb B, where it was stopped by closing the clamp at D. The point C could be observed through a window (indicated by the dotted circle) cut in the insulation on both sides of the tube and illuminated from behind by a 60-watt lamp. As soon as the bulb of ester exploded (generally two or three seconds after reaching C) the stop clock was started and the mercury in M_1 was brought level with that at C by opening T_1 and by manipulating the clamp at D and the taps T_4 and T_5. As no attempt was made to determine the initial pressure in B the latter operation could be

carried out at leisure. At this point the stabilizing volume V_1, which was closed during the evacuation of B, was connected to the system to facilitate pressure adjustments.

The reaction was followed at constant volume by balancing the manometer C-M_1 (also illuminated from behind) and reading the pressure on the manometer M_2 at measured intervals of time. Fine adjustment of the amount of mercury in C-M_1 was accomplished by means of subsidiary screw clamps on the rubber tube at D, while an accurate balance was obtained by the use of a sighting level consisting of a long straight 3-cm. glass tube almost filled with water, and adjustably fixed in a horizontal position about one foot from the manometer. This tube, adjusted until the air bubble remained in its middle, served as a convenient reference in balancing the manometer. M_2 and M_3 were backed by calibrated mirror scales graduated in millimetres.

To obtain the true pressure of the reaction, the vapor pressure of mercury at the temperature of B must of course be subtracted from all pressures registered on M_2. That the mercury surface at C exerts a pressure equal to that in the vapor jacket, in spite of the chance for heat leak through the mercury column below it, was confirmed by an experiment in which the vapor pressure of mercury was determined from 220° to 280° C. With B completely evacuated and the mercury surface held at C the readings of manometers M_2 and M_3 agreed within 1 mm. over the temperature range investigated. This pressure was attained within about two minutes of raising a cold mercury surface to C.

After a run was over all the mercury to the left of the clamp D was removed from the apparatus in order to avoid contamination of the next experiment by entrained reaction products, the cold tubing below C was wiped free of liquid and broken glass with clean cotton swabs, and the whole apparatus was thoroughly evacuated and flushed out several times with dry air. The reaction chamber was kept hot and the tubing below it was heated with a smoky flame during evacuation. When the tubing had cooled a fresh bulb of ester was introduced, the apparatus was again evacuated and the next run started.

As is pointed out in a later section, the nature of the formula by which the rate constant is calculated makes it necessary to determine the initial pressure with considerable accuracy if uniform constants are to be obtained over the whole run. It being impossible to determine the initial pressure directly with any certainty, recourse was had to extrapolation which in general gave quite satisfactory results. Due however to the fact that some uncertainty also existed in determining zero time, giving rise to a like uncertainty in the extrapolated initial pressure, more confidence is placed in the results calculated from the final pressure which is assumed to be twice the initial pressure. The final pressure had also to be determined by extrapolation since on account of a very slow secondary reaction, presumably the decomposition of acetaldehyde (9), the pressure never reached a stationary value. Each run was therefore continued for at least 24 hr. (at the lower temperatures for 48 hr.) and the true final pressure obtained by extrapolating the linear part of the pressure-time curve back to zero time. (By closing T_1 the apparatus could be left to itself

for an indefinite period.) This curve always straightened out after about 300 to 500 min. depending on the temperature and, at 268°C., had a slope equivalent to a pressure change of about 0.01 mm./min. The slope at the lower temperatures was much smaller giving a still more trustworthy extrapolation.

The velocity constants calculated from the initial pressure obtained in this way showed no drift even when the reaction was 90 to 95% complete, while those calculated from the directly extrapolated values of the initial pressure almost invariably showed a drift up or down toward the end of the reaction, depending on whether too high or too low a value was taken for the pressure at zero time. Constants calculated from initial pressures determined from the weight of the ester by the ideal gas laws were still more unsatisfactory, presumably on account of the large deviation of such a system from that of an ideal gas. All constants were calculated by a graphical method as explained in a later section.

Purification of the Ester

The ethylidene diacetate was obtained through the courtesy of the Shawinigan Laboratories Ltd.* It contained about 2% of acetic acid which was removed by washing with an excess of 0.3 *N* baryta, drying with $CaCl_2$ and fractionating twice *in vacuo*. The middle third, which was used in these experiments, analyzed 0.083% acetic acid and contained no other probable impurity such as acetic anhydride, vinyl acetate or acetaldehyde. It melted sharply at 17.5° C.

Products of the Reaction

As preliminary experiments had indicated the decomposition to be practically free from complicating side reactions, no attempt was made to analyze the contents of the reaction chamber *B* after a run. Instead a series of experiments was carried out in which the ester was passed through two metres of 5-mm. Pyrex tubing, in the form of a coil, enclosed in a piece of 2-in. iron pipe wound with nichrome wire. Temperatures were measured with a copper-constantan thermocouple in the centre of the furnace which was regulated by hand. Some 200 gm. of the ester was slowly introduced from a burette at as uniform a rate as possible, while the products were condensed in a CO_2-ether mixture and analyzed for acetaldehyde, acetic anhydride, acetic acid, vinyl acetate and ethylidene diacetate by standard methods.

Below about 250° C. the only products were acetaldehyde and acetic anhydride which were found in approximately equimolecular proportions and which corresponded, within the limits of error of the rather difficult analysis, to the amount of ester disappearing. The amount of the latter decomposed in any experiment depended of course on the temperature of the furnace and the rate of flow through the tube. No attempt was made to determine reaction velocities or equilibria by this dynamic method.

Above 300° C. a small quantity of acetic acid appeared which increased as

**The author wishes to take this opportunity of expressing his thanks to the Shawinigan Laboratories Ltd., and particularly to Mr. W. R. Elliot of that organization for the gift of two kilograms of purified ethylidene diacetate together with its analyses and particulars relating to its history and physical properties.*

the temperature was raised until at 400° C. it amounted to about 10 molecules per 100 molecules of ester decomposed. The acetic anhydride and acetaldehyde (plus its decomposition products) still being present in equimolecular proportions, it is concluded that the acid instead of being a product of the hydration from some source of the anhydride comes directly from the ester itself according to the equation,

$$CH_3CH(OOCCH_3)_2 \rightleftharpoons CH_3COOH + CH_2 = CHOOCCH_3.$$

A trace of vinyl acetate invariably accompanying the acetic acid lends support to this hypothesis. The fact that the vinyl acetate is never equivalent to the acetic acid is probably due to its immediate polymerization at the high temperature of the reaction tube. This would also account for the fact that the walls of the Pyrex coil became coated with a charred deposit during a high temperature run.

In the experiments above 350° C. a small quantity of permanent gas was also produced. This, collected over water and analyzed, was found to be CO and CH_4 in approximately equivalent quantities and is thus in all probability the result of some acetaldehyde decomposition (9).

Since in these dynamic experiments no product other than acetic anhydride and acetaldehyde was found below 300° C. it may be assumed that these are the only products of the static decompositions as well. That the equilibrium is shifted far enough to the right to make unnecessary any correction in the ordinary monomolecular equation for the velocity of the reverse reaction, is proved by the fact that the final pressure obtained by extrapolating, as described above, is always twice the extrapolated initial pressure within the limit of error of the later determination.

Since submitting the manuscript of this paper for publication the writer's attention has been called to a recent observation by Kassel (12) on the accelerated decomposition of acetaldehyde in the presence of mercury vapor. As this effect was observed to be measurable at temperatures as low as 177° C. the possibility is at once suggested that the true course of the ester decompositions described in this paper cannot be determined by pressure changes. In order to show that such a possibility is not a probability it is necessary to refer to several experiments which were carried out during the course of the present work and in which the effects mentioned by Kassel were not observed. Acetaldehyde vapor at pressures of from 10 to 30 cm. of mercury was admitted to the reaction chamber of the apparatus described above, the mercury was raised to the mark *C* and pressure readings were made from time to time over a period, in one case, of three days. In none of these experiments, all of which were carried out at 263° C., was the rate of pressure increase greater than that involved in the above-mentioned extrapolation, so that, except for a small uncertainty with regard to the final pressure, complications arising from the decomposition of acetaldehyde appear to be negligible.

Results

Calculation of Velocity Constants, etc.

The reaction being of the type $A \rightleftharpoons B + C$ the partial pressure of the ester (A)

at time t is equal to $2Po-P$, where Po is the total pressure at the beginning and P is the total pressure at time t, so that, under conditions where the equilibrium is shifted completely to the right, the monomolecular and bimolecular equations take the respective forms,

$$k_1 = \frac{1}{t}\, 2.303 \log \frac{Po}{2\,Po-P}$$

$$k_2 = \frac{1}{t}\, \frac{P-Po}{Po(2\,Po-P)},$$

i.e., if the graph of the expression $\frac{P-Po}{Po(2Po-P)}$ against time is a straight line the reaction is bimolecular, while if that of $\log \frac{Po}{2\,Po-P}$ against time gives a straight line the reaction is monomolecular. In Fig. 2 $\log \frac{Po}{2\,Po-P}$ is plotted against time for Runs 12 to 25 of Table I, which is a summary of all the experiments made to date in the apparatus described above. It is evident from the straight lines obtained that the reaction is definitely monomolecular with constants (sec^{-1})

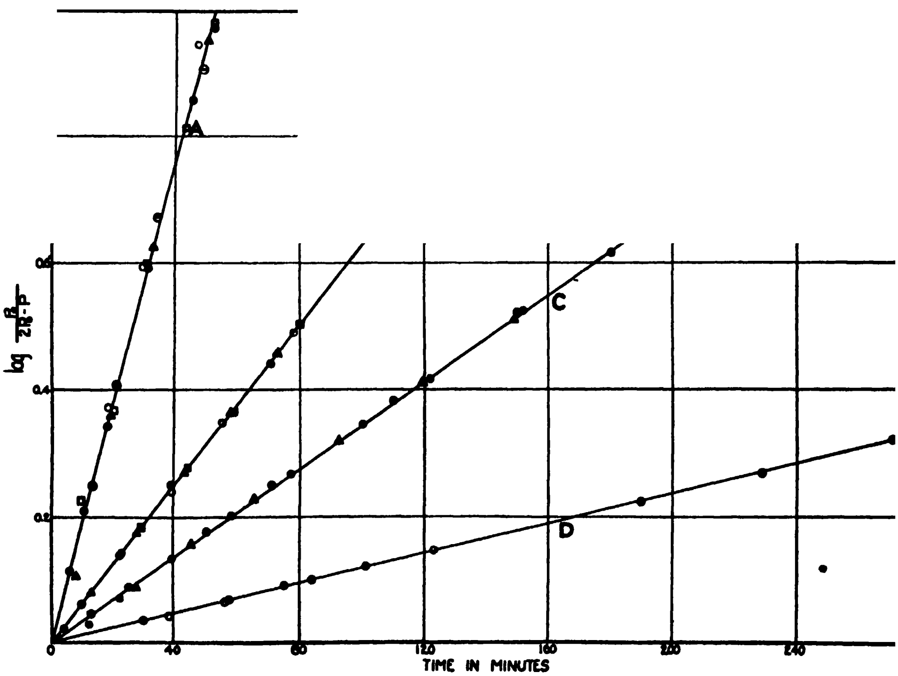


FIG. 2. *Plot of* $\log \frac{Po}{2Po\text{-}P}$ *against time (Runs 12-25).*

at the different temperatures equal to the slopes of the lines multiplied by $\frac{2.303}{60}$. The expression $\frac{P-Po}{Po(2\,Po-P)}$ plotted against time invariably gives a very pronounced curve.

In Runs 3 to 11 (Table I) the initial pressure was determined directly by extrapolation while Runs 12 to 25 were carried on for a time long enough to

extrapolate for the final pressure which was taken to be twice the initial pressure. For typographical reasons the data of the last set of runs only are plotted in Fig. 2. The first runs give results quite as good, until the reaction is about 80% complete, when some drift usually occurs on account of the above-mentioned uncertainty in the extrapolation. In each case the results were plotted on a large scale (x axis; 1 min. = 2.5 mm.: y axis; 1.0 = 500 mm.), the best straight line was drawn through the points obtained for all the runs at any one temperature and the rate constant was determined from the slope of the line. More weight was given to the points taken in the first half than to those in the last half of the reaction, on account of the magnification of the effect of the error in Po as $2Po - P$ decreases. The constants at the different temperatures are given in column 6 of Table I. They are considered to be correct to within at least 3%.

The fact that the time to half-value ($\log \frac{Po}{2Po-P} = 0.3$) at any temperature is independent of the initial pressure is well shown in Fig. 2 and affords conclusive proof of the strictly monomolecular character of the reaction. For example the weights of ester used in Runs 16 to 20 (Fig. 2, Curve A) were

TABLE I

EXPERIMENTAL RESULTS

Run No.	T °Abs.	Weight of ester, gm.	Po cm. Hg.	$\frac{Po}{\text{Wt. of ester}}$	k sec^{-1}	
3	515.0	0.1980	36.2	183		
4	515.0	0.2049	38.0	185	2.05×10^{-4}	
5	515.0	0.1036	19.9	191		
6	515.0	0.1083	21.0	194		
7	540.0	0.2192	44.3	202		
8	540.0	0.1747	35.8	205	9.14×10^{-4}	
9	540.0	0.1846	37.5	203		
10	529.0	0.2399	47.0	196	4.80×10^{-4}	
11	529.0	0.1343	26.3	196		
12	517.9	0.1364	35.00	256		Curve B○*
13	517.9	0.1578	40.15	254	2.41×10^{-4}	Curve B⊕*
14	517.9	0.1686	31.61	187		Curve B△
15	517.9	0.1660	31.10	187		Curve B□
16	535.8	0.1805	35.64	197		Curve A○
17	535.8	0.1881	36.55	194		Curve A⊕
18	535.8	0.0570	11.51	202	7.24×10^{-4}	Curve A△
19	535.8	0.0817	16.83	205		Curve A□
20	535.8	0.1312	26.20	200		Curve A⊖
21	507.4	0.1244	22.32	179		Curve C○
22	507.4	0.2616	46.28	177	1.32×10^{-4}	Curve C⊕
23	507.4	0.1101	19.80	179		Curve C△
24	493.0	0.2183	37.70	169	4.54×10^{-5}	Curve D○
25	493.0	0.2438	41.82	171		Curve D⊕

*NOTE:—*Surface-volume ratio increased about 20 times.*

varied from 0.057 to 0.188 gm. without bringing to light any appreciable trend in the velocity constant. It is to be noted that at $\log \frac{Po}{2Po-P} = 1.0$ the reaction is 90% complete.

As has already been mentioned it was found necessary to determine zero time by back extrapolation of the straight line obtained by plotting $\log \frac{Po}{2Po-P}$ against time. The zero so estimated was generally four to eight minutes after the bursting of the bulb and thus suggests the existence of an "induction period". This point is under investigation at present. The slope of the line, *i.e.*, the rate constant, is uninfluenced by this uncertainty with regard to zero time.

Column 5 gives the values of the ratio *Po*/weight ester. If the equilibrium were not shifted completely to the right this ratio should increase with decreasing pressure. A slight difference is in fact usually to be observed in the runs at any one temperature (*e.g.*, Runs 16 to 20) but as it is of the order of magnitude to be expected from deviations from the ideal gas laws it is probable that even at the higher pressures the ester is completely dissociated. On account of the small heat of reaction the equilibrium constant would not be expected to change very much with temperature.

Homogeneity of the Reaction

In Runs 12 and 13 the surface-volume ratio was increased about 20 times by filling *B* with short lengths of small Pyrex tubing held in place by a plug of glass wool above *C*. From Fig. 2 (Curve B Run 12, ○, Run 15, ⊕) it is evident that the velocity of the reaction is absolutely independent of the extent of the glass surface of the reaction chamber, *i.e.*, the reaction takes place homogeneously throughout the body of the gas.

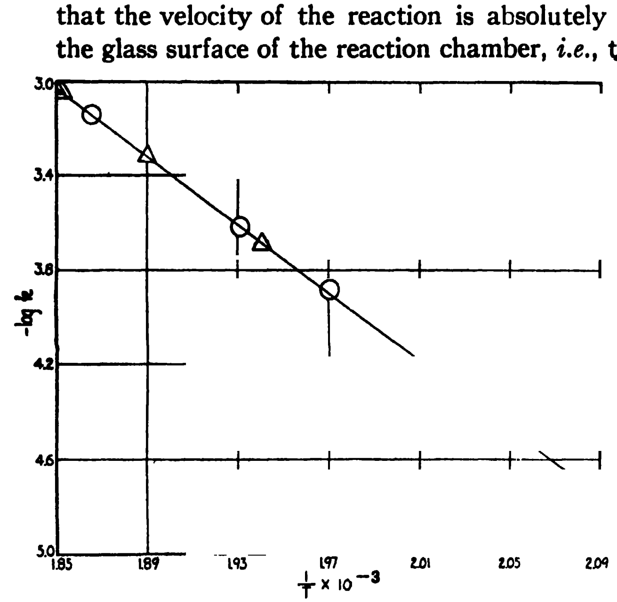


FIG. 3. *Graph of log k against 1/T. The circles represent the data of Runs 12 to 25 and the triangles those of Runs 3 to 11.*

After a few runs at the higher temperatures the walls of *B* become coated with a very thin brown deposit which can be washed off with alcohol and ether as thin flakes of film resembling collodion. This is probably polymerized vinyl acetate liberated with acetic acid in the secondary reaction already discussed. Between Runs 18 and 19 the apparatus was taken down and *B* was thoroughly cleaned with alcohol, ether and hot chromic acid solution. No difference in the velocity of the runs before and after cleaning could be detected.

The Energy of Activation

Fig. 3 gives the usual graph of log k against $\frac{1}{T}$. The straight line obtained shows that the reaction obeys the Arrhenius equation and gives a heat of activation of 32,900 cal./mol. The circles represent the data of Runs 12 to 25 and the triangles those of Runs 3 to 11. The velocity constant at any temperature is given by the equation,

$$\ln k = 23.74 - \frac{32900}{RT}.$$

The energy of activation and the absolute temperature at which the decomposition attains a given rate, agree well with the parallelism exhibited by the known examples of first order reactions (5).

Discussion

The fact that these esters decompose by the monomolecular mechanism is in harmony with the suggestion of Hinshelwood (7) and others, that first order reactions are characteristic of complicated molecules which can use their large number of internal degrees of freedom as energy reservoirs, and so be temporarily independent of activating collisions in the sense that the latter are necessary for bimolecular reactions. Moreover although the decomposition is endothermic the heat of reaction is small, and hence from thermochemical considerations a large heat of activation is not required (5). A monomolecular decomposition above a certain limiting pressure is therefore to be expected.

The generally accepted picture of the monomolecular mechanism in the gaseous state is based on the suggestion of Lindemann (13) that there exists a time lag between activation and reaction, which is large compared to the time between molecular collisions, so that the majority of molecules activated at any instant suffers deactivation and only a small constant fraction succeeds in reacting. In other words, the essentially bimolecular processes of collisional activation and deactivation take place at a rate greater than that at which activated molecules are lost by reaction. The fraction of molecules activated at any instant being thus in excess of the fraction reacting at that instant and independent of the pressure of the reactant, the reaction velocity is also independent of the pressure of the reactant and kinetically the decomposition follows the monomolecular law. The maintenance of this excess of activated molecules is therefore a result of processes characteristic of pressures at which the "mean free time" is small compared to the average time between activation and reaction, and it is to be expected that as the pressure is lowered a point will be reached where these two time intervals approach equality, with the result that an increasing fraction of activated molecules will be removed by reaction and the velocity constant will begin to fall. That this theory gives essentially the correct picture is evinced by the fact that the majority of the known monomolecular reactions do show just such a decrease in velocity as the pressure is lowered beyond a certain value.

This limiting pressure is a specific property of the molecule in question, being dependent on the magnitude of the time lag between activation and reaction

or upon the number of internal degrees of freedom involved in the activation process. At present the scarcity of data concerning monomolecular reactions allows little to be predicted with regard to this number, beyond the fact that it apparently increases with the complexity of the reaction and the molecule. Highly specific structural effects have been observed (3) but as yet the data are insufficient to warrant any generalizations whatsoever.

It is thus impossible to say whether or not the esters of this series possess too complicated an activation mechanism to show the predicted decrease in velocity constant at measurable pressures. If this decrease, which is now being looked for, does occur before the pressure becomes too small to measure, it is possible that this series of reactions will furnish interesting data with regard to the effect of molecular structure on the number of internal degrees of freedom associated with the activation process. Such data are particularly desirable in view of the fact that the majority of the monomolecular reactions described in the literature are more or less complex "pyrolytic" decompositions which involve the rupture of several bonds at once, are apt to undergo considerable changes in mechanism with alterations in molecular structure and which in general are too complicated to furnish much information with regard to the details of activation or reaction. The comparative simplicity, therefore, of the decomposition of these esters, together with the fact that the reaction mechanism appears to remain unchanged throughout the series, are important considerations in connection with the theoretical significance of the velocity data.

The system being of the type $A \rightleftharpoons B + C$ the existence of a homogeneous reverse reaction and the reality of a statistical equilibrium in the gaseous state are of particular interest in connection with the "dreierstoss" theory. Since Herzfeld (4) first pointed out that the reaction of two atoms to form a molecule must be a termolecular or a wall reaction in order that the freshly formed molecule be deactivated and immediate decomposition prevented, a tendency has existed to extend this idea to include all bimolecular association reactions. In a recent discussion of the possibility of such reactions Kassel (11) has argued that, although none of the monomolecular reactions so far investigated lead to a measurable equilibrium, the existence of the time lag demanded by Lindemann's hypothesis between activation and reaction removes such cases from the restriction of the "dreierstoss" theory. He states, "A complex molecule once formed at a binary collision may then be stabilized at any subsequent collision within this period (of time)."

While it is not quite clear how such a molecule can be formed in the first place, since at the moment of incipient formation it would be expected to possess just that energy distribution which ordinarily occurs only after the lapse of the time interval in question, it nevertheless seems probable from Kassel's arguments that such reactions can and do occur. It is evident, however, that under the conditions of the foregoing experiments the velocity of the reaction between acetaldehyde and acetic anhydride is, like the reverse of other first order changes, negligible in comparison with that of the ester decom-

position. Whether or not other conditions of pressure and temperature will bring about a measurable equilibrium and enable the kinetics of the reverse reaction to be examined remains to be seen. This point, which is of interest also in connection with the readily determined liquid phase equilibrium, is being investigated.

References

1. Coffin, C. C. and Maass, O. Can. J. Research, 3: 526-539. 1930.
2. Coffin, C. C. and Maass, O. Can. J. Research, 3: 540-542. 1930.
3. Glass, J. V. S. and Hinshelwood, C. N. J. Chem. Soc. 1804-1814. 1929.
4. Herzfeld, K. F. Z. Physik, 8: 132-136. 1922.
5. Hinshelwood, C. N. Kinetics of chemical change in gaseous systems. 2d. ed. Oxford. 1929.
6. Hinshelwood, C. N. Proc. Roy. Soc. Lond. A, 113: 230-233. 1927.
7. Hinshelwood, C. N. Proc. Roy. Soc. Lond. A, 114: 84-97. 1927.
8. Hinshelwood, C. N. and Askey, P. J. Proc. Roy. Soc. Lond. A, 115: 215-226. 1927.
9. Hinshelwood, C. N. and Hutchison, W. K. Proc. Roy. Soc. Lond. A, 111: 380-385. 1926.
10. Hinshelwood, C. N. and Thompson, H. W. Proc. Roy. Soc. Lond. A, 113: 221-229. 1927.
11. Kassel, L. S. J. Am. Chem. Soc. 53: 2143-2147. 1931.
12. Kassel, L. S. J. Phys. Chem. 34: 1166-1173. 1930.
13. Lindemann, F. A. Trans. Faraday Soc. 17: 598-599. 1922.
14. Ramsperger, H. C. J. Am. Chem Soc. 49: 912-916. 1927.
15. Ramsperger, H. C. J. Am. Chem. Soc. 49: 1495-1499. 1927.
16. Ramsperger, H. C. J. Am. Chem. Soc. 50: 714-721. 1928.
17. Ramsperger, H. C. J. Am. Chem. Soc. 51: 2134-2143. 1929.
18. Ramsperger, H. C. and Leermakers, J. A. J. Am. Chem. Soc. 53: 2061-2071. 1931.

THE OXIDATION OF ACETALDEHYDE[1]

By W. H. Hatcher[2], E. W. R. Steacie[3] and F. Howland[4]

Abstract

Acetaldehyde, when freshly distilled, suffers immediate oxidation on coming into contact with oxygen or air. A compound is produced which in aqueous solution behaves as an organic peracid. This has a pronounced effect upon the subsequent gas-phase oxidation of the acetaldehyde.

Introduction

During a study of the oxidation of acetaldehyde in the vapor phase, the obviation of certain difficulties which were encountered has uncovered the susceptibility of this compound to attack by oxygen, even at room temperature. In this communication it is not proposed to discuss the results of the systematic study of the gas-phase oxidation of acetaldehyde but merely to touch on those results which bear on the susceptibility of the compound to low temperature oxidation.

Bowen and Tietz (1) have recently stated that acetaldehyde and oxygen in ultra-violet light produce peracetic acid. It has long been known that benzaldehyde behaves similarly, but in the past insufficient attention has been paid to such behavior on the part of aliphatic aldehydes. It has also been observed, during the course of measurements on the rate of polymerization of acetaldehyde (2), that a serious change in the velocity of polymerization occurs if the material is exposed to the air. The following pages will serve to emphasize the necessity of the absolute exclusion of air or oxygen from acetaldehyde during its preparation for subsequent experimental work.

Experimental

The course of the high temperature oxidation of acetaldehyde was followed by the rate of change in pressure of a mixture of acetaldehyde and oxygen in a system which permitted but a negligible change in volume. The gases were first mixed in a bulb and were then let into the reaction flask, where the pressure was read by means of a capillary mercury manometer.

The oxygen used was obtained from a cylinder and was not purified except by drying over phosphorus pentoxide. The acetaldehyde was prepared from high grade paraldehyde by distillation with a few drops of concentrated sulphuric acid.

Preliminary experiments were carried out from 175 to 250°C. with partial pressures of acetaldehyde from 3 to 12 cm., together with one to three times as much oxygen. It was found that at these temperatures the reaction was accompanied at first by a decrease in the total pressure. After reaching a minimum the pressure then rose again and a constant value was finally reached

[1] *Manuscript received October 27, 1931.*
Contribution from the Department of Chemistry, McGill University, Montreal, Canada.
[2] *Associate Professor of Chemistry, McGill University.*
[3] *Assistant Professor of Chemistry, McGill University.*
[4] *Graduate Assistant in Chemistry, McGill University.*

which was considerably higher than the initial pressure. In these experiments excess oxygen was always used.

In these preliminary experiments it was not possible to obtain results for the rate of oxidation which were at all consistent. In addition, explosions sometimes occurred a few seconds after the mixture of gases reached the reaction flask. At the same time difficulty was encountered in keeping the reaction flask, as well as the tubing leading to it, free from a white crystalline deposit. On examination this deposit proved to be a compound of mercury, since it yielded mercury on decomposition.

Several cc. of mercury were therefore introduced into the reaction flask and oxidation experiments were carried out at 120° C. After each experiment a considerable quantity of this white material was found on the mercury surface. A large quantity of the material was then prepared under similar conditions. It seemed probable that it should be a mercurous or mercuric salt of either formic or acetic acid. A comparison of many properties proved that the substance in question was mercurous acetate.

The apparatus was therefore modified and the reactants were prevented from coming into contact with mercury by means of an air buffer above the manometer surface. Thereafter no deposit formed in the tubing, and explosions never occurred.

After the above changes, however, it was still impossible to obtain consistent results. Examination of the results indicated that the velocity of the oxidation and the total pressure drop were apparently decreased by allowing the mixture of oxygen and acetaldehyde to stand for any appreciable length of time in the mixing bulb before being sent into the reaction flask.

For example, at 118° C. with a partial pressure of acetaldehyde of 13.7 cm., and a partial oxygen pressure of 20.5 cm., the maximum diminution in pressure was 92% of the partial pressure of acetaldehyde if the reactants were admitted to the bulb immediately after mixing. If, however, the reactants were allowed to stand for 30 min. before admission to the reaction flask the total pressure drop was only 83% of the initial partial pressure of acetaldehyde. If the reactants stood for two hours before admission the pressure drop diminished still further to 69% of the initial pressure of acetaldehyde.

These discrepancies can be explained if the oxidation of acetaldehyde proceeds to a marked extent at room temperature. If this were so the calculated value for the initial pressure of acetaldehyde would be too high. The calculated pressure drop, expressed in terms of acetaldehyde pressure, would therefore be too low as is observed experimentally.

It seemed reasonable to assume that if as short a contact period for acetaldehyde and oxygen as 30 min. could have such a large effect, the oxidation of acetaldehyde might be appreciable even during its distillation, or when it came into contact with air for a brief period. This assumption is borne out by the fact that Bowen and Tietz (1) found that liquid acetaldehyde absorbs oxygen quite rapidly at room temperature. In the gas-phase oxidation the maximum pressure drop encountered in the initial phase of the reaction was never

greater than the initial partial pressure of acetaldehyde. This would indicate that the reaction occurring is

$$CH_3CHO + O_2 \rightleftharpoons (CH_3CHO.O_2),$$

and that some oxide or peroxide of acetaldehyde is formed. Such a compound would be unstable and would give an explanation of the explosions encountered when acetaldehyde-oxygen mixtures came into contact with mercury.

In view of these conclusions a few drops of acetaldehyde which had been freshly distilled in a thoroughly cleaned glass apparatus were dropped into a neutral 10% solution of potassium iodide. Iodine was liberated at once in sufficient quantity to produce a deep yellowish-brown color. This reaction, a test for a peroxygen compound, was obtained immediately after the distillation of acetaldehyde, as well as several hours later. A mixture of acetaldehyde vapor and oxygen gave the same result when shaken with a solution of potassium iodide.

In order to obtain acetaldehyde free from a trace of any peroxygen compound it was necessary to distil it in the absence of air and to keep it out of contact with air thereafter. Acetaldehyde was therefore distilled in the presence of carbon dioxide. In these distillations dry, oxygen-free, carbon dioxide was circulated through an air-tight distillation apparatus for two hours. Acetaldehyde was then run into the distilling flask from a dropping funnel. Potassium iodide solution which had been boiled to remove dissolved oxygen was simultaneously run into the ice-cooled receiver of the distillation apparatus. Twenty drops of acetaldehyde dropping directly from the condenser failed to produce any color change in the solution of potassium iodide. If, however, air was momentarily let into the apparatus, the next five drops of acetaldehyde produced a deep yellow color. Apparently then, a momentary contact with air is sufficient to produce an appreciable quantity of some peroxygen compound.

In a further experiment oxygen was bubbled through a colorless solution of potassium iodide and acetaldehyde which was kept cold in an ice bath. After 30 min. of such treatment a slight yellow color was detected, which deepened somewhat when the solution was warmed to room temperature. However, the color never approximated in depth that produced by five drops of acetaldehyde which had come into contact with air before reaching the solution.

The instantaneous liberation of iodine from a neutral solution of potassium iodide is, of course, characteristic of a so-called peracid. On the other hand, an organic peroxide requires time to hydrolyze to the peracid before any coloration becomes apparent. The acetaldehyde-oxygen complex would therefore seem to possess the characteristics of an organic peracid.

It is not difficult to formulate a mechanism for the above reactions, but it is deemed advisable to defer this until the completion of the investigation of the gas-phase oxidation. In addition, investigations are under way on the properties of oxygen-free acetaldehyde.

References

1. Bowen, E. J. and Tietz, E. L. Nature 124: 914. 1929.
2. Hatcher, W. H. and Brodie, B. Can. J. Research, 4: 574-581. 1931.

THE ALKALOIDS OF *SENECIO* SPECIES

I. THE NECINES AND NECIC ACIDS FROM *S. RETRORSUS* AND *S. JACOBAEA*[1]

By Richard H. F. Manske[2]

Abstract

A new alkaloid, *retrorsine*, has been isolated from *Senecio retrorsus* of South African origin. Analysis of the free base and its methiodide together with its hydrolytic products point to the empirical formula, $C_{18}H_{25}O_6N$. Hydrolysis yields a new base, $C_8H_{13}O_2N$, termed *retronecine* together with an acid, *retronecic acid*, isolated as the monolactone, $C_{10}H_{14}O_5$. The base is tertiary, contains one hydroxyl (benzoyl derivative) and probably a ketonic group. The preparation of the di-*p*-phenylphenacyl derivative of the acid proves its dibasicity. The alkaloid, jacobine, from *S. jacobaea* has been isolated in a state of purity and probably has the empirical formula $C_{18}H_{25}O_6N$, although this is only partly confirmed by analysis of the hydrolytic fragments. The necine derived from jacobine is shown to be identical with retronecine.

An examination of *S. aureus* failed to show the presence of an alkaloid in tractable amounts. A system of simplified nomenclature to designate the *Senecio* alkaloids and their hydrolytic products is suggested.

The *Senecio* species belong to the natural order Compositae and enjoy the distinction of being the only members of this order which have been shown to contain well-defined alkaloids in appreciable quantities. It frequently occurs that different species belonging to the same botanical genus elaborate a group of alkaloids of similar if not identical structure although the associated minor alkaloids may vary considerably. As an example the cinchona alkaloids serve well, in that they are confined entirely to the suborder Cinchonoideae of the N.O. Rubiaceae which is particularly rich in alkaloids, and each suborder is characterized by its own group of closely related bases. In the N.O. Ranunculaceae the occurrence of the characteristic aconitines is confined exclusively to the genus *Aconitum*, and no species of this genus has been found to be devoid of alkaloids. On the other hand some alkaloids seem to represent the final stages of a diversity of phytochemical processes and no better example than berberine, which occurs in five different natural orders, could be cited.

Several species of *Senecio*, *e.g.*, *S. kämpferi* (4), appear to be devoid of alkaloids and an examination of *S. aureus* has now shown that if any is present the amount is excessively small.

It seemed of interest therefore to investigate in some detail a number of *Senecio* species and to ascertain whether or not the alkaloids from the various sources are closely related structurally, if indeed not identical in some cases. Added interest is attached to the investigation because of the fact that the alkaloids in question appear to be rather toxic and produce cumulative effects when ingested by livestock in small doses over extended periods. Winton disease, so common in the Maritime Provinces, has been shown to be caused by the Giant Ragwart (*S. jacobaea*) and a similar malady due to *S. latifolius* and to *S. burchelli* has been responsible for many losses among livestock in

[1] *Manuscript received November 9, 1931.*
Contribution from the National Research Laboratories, Ottawa, Canada.

[2] *Associate Research Chemist, National Research Laboratories.*

South Africa. The common groundsel (*S. vulgaris*) of European origin is not known to be toxic although it was the first to be examined chemically. From the latter, Grandval and Lajoux (3) isolated two alkaloids in small amounts. Only one, however, was obtained in sufficient quantity for analysis.

The only other contribution to our knowledge of *Senecio* alkaloids which deserves mention is that of Watt (5) dealing with *S. latifolius*. Two well-defined alkaloids, senecifoline ($C_{18}H_{27}O_8N$) and senecifolidine ($C_{18}H_{25}O_7N$) were shown to be present, the latter in traces only. Senecifoline on treatment with potassium hydroxide in absolute alcohol was readily hydrolyzed to an acidic and a basic fragment. The latter, termed senecifolinine, was isolated as the hydrochloride (m.p. 168° C.) and on the basis of analysis was given the formula $C_8H_{11}O_2N$. The acid (m.p. 198° C.), termed senecifolic acid, as the result of combustion and titration, was given the formula $C_{10}H_{16}O_6$, and was assumed to be a dibasic acid. Watt pointed out that the elements of a molecule of water were not taken up in this hydrolysis, a point which was admittedly obscure.

Owing to the possible complexity involved in naming the parent alkaloids together with the hydrolytic products of each, it seems desirable to simplify the nomenclature somewhat, and it is proposed to coin the generic name, *necine*, for the basic hydrolytic product and to reserve the name, *necic* acid, for the acidic fragment.

The present memoir contains an account of a preliminary examination of the alkaloids of *S. retrorsus* and of *S. jacobaea*. The former plant material was of South African origin and the author wishes to express his indebtedness to Dr. E. Percy Phillips, Principal Botanist of the Union of South Africa (Pretoria) who generously supplied the material. There appears to be some doubt regarding the specific name owing to the uncertainty and confusion surrounding the identification and naming of the *Senecio* species of South African habitat, and until this point has been clearly settled the exact botanical origin must be left undecided. The choice, however, is particularly fortunate on account of the comparatively high content of alkaloid and furthermore the material was probably homogeneous since a thorough search failed to reveal more than one base.

The alkaloid crystallizes with great facility from a variety of solvents and in common with the others derived from *Senecio* species has no sharp melting point. The most characteristic derivative is the methiodide which crystallizes from hot water in stout brilliant prisms. Analytical figures of the base and of the methiodide are in good agreement with the formula $C_{18}H_{25}O_6N$, which is amply confirmed by analysis of the hydrolytic products. Inasmuch as the substance appears to be new the name *retrorsine* is proposed for it.

Hydrolysis yielded the necine, $C_8H_{13}O_2N$, isolated as the hydrochloride, for which the name *retronecine* is suggested, together with an acid, $C_{10}H_{16}O_6$, now termed *retronecic* acid. Retronecine hydrochloride crystallizes in deep tetragonal plates melting at 164° C.* and on the basic of its melting point and formula appears to differ from Watt's senecifolinine hydrochloride. Benzoylation with excess benzoyl chloride in the presence of potassium carbonate yields

**All melting points are corrected.*

only a monobenzoyl derivative which is still basic and yields a homogeneous methiodide. It therefore appears that the nitrogen is tertiary and that one, and only one, oxygen is present as a hydroxyl group. Some evidence that the remaining oxygen atom is present as a ketonic group is found in the fact that on treatment with piperonal and alkali an intense yellow color is developed. This observation further indicates that the groups $—CH_2.CO—$, or $—CH_2.CO.CH_2—$ may be present.

Retronecic acid forms a dipotassium salt which is sparingly soluble in absolute alcohol and crystallizes almost quantitatively from the reaction mixture during hydrolysis. The potassium salt on treatment with *p*-phenylphenacyl bromide (2) yields the corresponding diphenacyl derivative, an observation which sufficiently proves the acid to be dibasic. It is, however, not likely that the two carboxyl groups are concerned in the esterification of retronecine to form the parent alkaloid. Such a supposition would require two hydroxyls in retronecine which are probably not there, and the presence of at least an eight membered ring containing two ester groups,—an unlikely constitution. The latter supposition furthermore is not consistent with the observation that only one molecule of water is taken up in the hydrolysis of retrorsine. There remains the logical alternative therefore that only one hydroxyl is concerned in the esterification and that the remaining one functions as a lactone. This view is largely confirmed by the observation that retronecic acid loses a molecule of water with extreme ease. It suffices to heat the acid for a short time on the water bath or to evaporate its solution in ethyl acetate and the resulting product, almost certainly the monolactone, crystallizes readily.

Although it has been known for some time that one or more alkaloids are present in *S. jacobaea* (1) the base or bases have not been satisfactorily characterized. It has now been possible to examine the base in some detail due to the courtesy and valued co-operation of Mr. R. R. Hurst, Plant Pathologist in charge at Charlottetown, P.E.I., who on numerous occasions supplied material of authentic botanical origin.

The alkaloid crystallizes with great facility but unfortunately the total content of the plant is exceedingly low. Analysis of the base and its methiodide point to the expirical formula $C_{18}H_{23}O_5N$, and this is partly confirmed by analysis of the hydrolytic products. The name, *jacobine*, indicative of its botanical origin is proposed for the alkaloid.

The necine obtained by the procedure already referred to is identical with retronecine but the necic acid, for which the name *jaconecic* acid is coined is different from retronecic acid and furthermore does not appear to be identical with senecifolic acid (5). Analyses give values in substantial agreement with the formula $C_{10}H_{16}O_6$, *i.e.*, the same as senecifolic acid. This formula is given with reserve and it is proposed to continue the investigation when more material is available.

During the isolation of the alkaloids from the above two species a number of other products were encountered, an examination of which is reserved for a later opportunity. Two substances, however, may be mentioned in passing.

The aqueous acid solution obtained from *S. retrosus* yielded to chloroform extraction a mixture from which one substance crystallized with great facility in large colorless hexagonal plates. Except for the analytical data which indicate $C_{12}H_{16}O_7$, its properties have not been investigated.

The second substance has been obtained only from *S. jacobaea* as a phenylhydrazone, melting at 178° C. Analytical figures are in good agreement with $C_7H_6O_2 : N.NH.C_6H_5$, and although the melting point of protocatechuic aldehyde phenylhydrazone is also close to 178° C. (6), a mixture of the two begins to sinter some 20° C. lower. The substance, prior to combination with phenylhydrazine, is not extracted appreciably from aqueous solution by chloroform.

Experimental

Retrorsine

Finely ground leaves and stems (6.3 kilos) of *Senecio retrorsus* were extracted in a Soxhlet extractor with purified methanol until no further extraction took place. Citric acid was added to the extract until it was strongly acid to litmus and the greater portion of the solvent was removed on a steam bath. Water was added to the residue until no further precipitation ensued and the resin was allowed to settle for several days. The supernatant liquid was then filtered with suction and the residue thoroughly washed with water. The filtrate was then stirred up with a small quantity of charcoal and the small amount of methanol still remaining was removed on a steam bath in a current of air. The cooled mixture was filtered through a layer of filtercel and the filtrate extracted with chloroform, until no further color was removed.

The combined chloroform extracts on clarification and evaporation yielded a brown viscous residue which rapidly deposited large hexagonal crystals. The incorporation of a little alcohol facilitated the removal of the mother liquor at the pump. The cautiously washed crystals were redissolved in chloroform, the solution evaporated to a small volume and treated with much ether. The clear supernatant solution was decanted from a small amount of gummy precipitate, and evaporated to a small volume. While still warm the substance began to separate in the colorless characteristic plates. The mixture was again recrystallized by solution in a small volume of hot chloroform and addition of ether. As thus obtained the substance begins to sinter at 175°, shrinks considerably at 182-186° and melts to a clear liquid at 190° C. Analysis: Calcd. for $C_{12}H_{16}O_7$; C, 52.94; H, 5.88%; mol. wt. 272. Found: C, 53.58, 53.80; H, 6.10, 6.18%; mol. wt. 279, 277 (Rast).

The aqueous solution from the above chloroform extract was again filtered through a layer of charcoal to remove a small amount of dark resin which had separated during the chloroform extraction. After the removal of the chloroform the mixture was made distinctly alkaline with potassium hydroxide and allowed to remain at room temperature for several days, during which time an alkaloid gradually separated in crystalline form. This was filtered off and the filtrate exhaustively extracted with chloroform. It had previously been observed that the base which crystallized directly was identical with that which

was removed by chloroform extraction, so the chloroform extract was added to the crystalline material and the mixture heated until solution was complete, filtered with the aid of charcoal, and evaporated to incipient crystallization. A small volume of methanol was added and crystallization allowed to proceed to completion at a low temperature. The base was filtered off and washed with cold methanol. The filtrate and washings were combined, acidified with citric acid, diluted with water and the remaining methanol boiled off. The deposited resin was filtered off through charcoal and some further impurities removed by extraction with chloroform. The alkaloid was removed by means of chloroform from the basified solution and crystallized as described above. The final mother liquor on concentration to a small volume deposited some more base which was combined with the first two crops. A repetition of the solution in acid and removal of impurities yielded a further small amount of the same base. The total yield of this crystalline product, which melted at about 212° C., darkening taking place at 208° C., was 81.8 gm. (1.3%).

The final mother liquors which yielded no further crystalline material, due no doubt to the accumulation of impurities, was treated in chloroform solution with methyl iodide. A rapid separation of a methiodide took place and recrystallization of the product yielded the characteristic substance shortly to be described. It therefore appears that no other base is present in appreciable quantity in *S retrorsus.*

Purification of the above base was effected in various ways, namely, recrystallization from acetone, from chloroform, from a large volume of methanol, and in no case separation into two or more substances could be effected. The crystals when slowly formed occur in short, stout tetragonal prisms one end of which is frequently developed obliquely. The melting point of the repeatedly recrystallized substance is indefinite at 214-215° C. with darkening and frothing, sintering taking place a few degrees lower. It gives no coloration with Ehrlich's reagent but an observation of undoubted structural significance is that the decomposed melt gives an immediate reddish-purple tint. The same is true of the overheated retronecine hydrochloride and undoubtedly indicates a latent pyrrol nucleus, which requires only dehydration to pass into the aromatic type. Analysis: Calcd. for $C_{18}H_{25}O_6N$; C, 61.54; H, 7.12; N, 3.99%. Found: C, 60.95; H, 7.14; N, 4.01%.

This analysis is the mean of three concordant combustions. It will be observed that the value for carbon is slightly low, and this has also been found to be the case with jacobine and the base from *S. vulgaris.* The derivatives and hydrolytic products however give more satisfactory figures.

Retrorsine Methiodide

When methyl iodide is added to a chloroform solution of retrorsine containing a small amount of alcohol a rapid separation of the methiodide in crystalline form takes place. The product is soluble with difficulty in hot alcohol and moderately soluble in hot water from which it crystallizes in brilliant stout prisms with blunt pyramidal terminations. It darkens at 256° C. and swells up and chars completely at 266° C. Analysis: Calcd. for $C_{19}H_{28}O_6IN$; C, 46.25; H, 5.68; N, 2.84; I, 25.76%. Found: C, 46.51; H, 5.69; N, 2.71, I, 26.05%.

Retronecine and Retronecic Monolactone

A solution of 2.5 gm. of retrorsine in 75 cc. of hot absolute alcohol was treated with 3 gm. of potassium hydroxide dissolved in 2 cc. of water. In the course of about 10 min. the potassium salt of retronecic acid began to separate in fine colorless needles. The mixture was allowed to remain overnight, then heated to boiling, cooled, and the separated salt filtered off and washed with cold absolute alcohol.

The filtrate was made just acid to Congo red with conc. hydrochloric acid, some potassium chloride filtered off and the alcohol evaporated from the filtrate. The dry residue was thoroughly washed with hot ethyl acetate which removed a small amount of retronecic acid. The slightly colored crystalline residue was freed of potassium chloride by several recrystallizations from absolute alcohol. In the final purification for analysis a concentrated alcoholic solution was treated with ethyl acetate until a considerable turbidity had developed. The mixture was rapidly filtered, the filtrate evaporated somewhat and cautiously treated with acetone. Retrorsine hydrochloride as thus obtained consists of transparent tetragonal plates, containing no water of crystallization. It melts sharply at 164° C. to a clear colorless liquid. Analysis: Calc. for $C_8H_{13}O_2N.HCl$: C, 50.14; H, 7.31; N, 7.31; Cl, 18.52%. Found: C, 50.26; H, 7.44; N, 7.00; Cl, 18.52%.

The retronecic monolactone is conveniently obtained from the above potassium salt by treatment with conc. hydrochloric acid until acid to Congo red, evaporation to dryness on the steam bath in a current of air, and extraction with hot ethyl acetate. The extract on treatment with charcoal and evaporation to a small volume yielded the crystalline substance on cooling. When slowly crystallized from ethyl acetate it was obtained in elongated needles generally terminated on one end with a pyramid and on the other with an oblique face. It is sparingly soluble in cold ethyl acetate and acetone, and almost insoluble in ether. No solvent is lost on heating. The pure substance melts at 186° C. and solidifies on cooling. The melting point of the resolidified melt is a few degrees lower. Analysis:— Calcd. for $C_{10}H_{14}O_5$; C, 56.08; H, 6.54%. Found: C, 55.90; H, 6.68%.

Benzoyl-retronecine Hydrochloride

A mixture of retronecine hydrochloride and powdered potassium carbonate was treated with an excess of benzoyl chloride in chloroform, and gently heated under reflux for 30 min. The chloroform solution was washed with water and the base extracted with dilute citric acid solution. The base was regenerated by the addition of alkali and extracted with chloroform. The extract was clarified over potassium carbonate and the solvent removed. Benzoyl retronecine as thus obtained consisted of a colorless viscous syrup readily soluble in ether. It failed to crystallize in contact with a number of solvents.

The hydrochloride in the amorphous state is readily soluble in acetone but when once obtained crystalline dissolves with difficulty. It was recrystallized by seeding a solution in acetone with a nucleus that had separated from another preparation. It consists of colorless needles, melting at 151° C.

Analysis:— Calcd. for $C_{16}H_{17}O_3N.HCl$; C, 60.92; H, 6.09; N, 4.74; Cl, 12.00%. Found: C, 60.56; H, 6.37; N, 4.85; Cl, 11.67%.

Benzoyl-retronecine Methochloride

The free base on treatment with methyl iodide in chloroform yielded an immediate precipitate of the methiodide which however was not obtained crystalline. It was readily soluble in acetone. The methiodide was converted into the methochloride by heating in aqueous solution with an excess of silver chloride. The filtrate was evaporated to a small volume. Crystallization occurred while the solution was still warm, and was hastened by the addition of acetone. The substance was recrystallized from alcohol-ether. As thus obtained it consists of stout, irregular plates, melting at 128° C. Analysis:— Calcd. for $C_{16}H_{20}O_3NCl$; C, 62.04; H, 6.47; N, 4.52; Cl, 11.46%. Found: C, 60.40; H, 6.72; N, 4.40; Cl, 11.24%.

Di-p-phenylphenacyl Ester of Retronecic Acid

The dried potassium salt of retronecic acid was accurately weighed and treated with exactly two moles of *p*-phenylphenacyl bromide in sufficient 90% alcohol to effect complete solution when hot. In the course of heating for three hours some of the ester had crystallized. Water was cautiously added until the incipient turbidity just disappeared on mixing. When crystallization was complete the solid was filtered off, washed with cold alcohol in which it is sparingly soluble and recrystallized twice from acetone. The latter operation was conveniently effected by evaporating the charcoaled solution to a small volume, adding alcohol and slowly evaporating most of the remaining acetone. Slow cooling yielded a mass of minute needles which melt sharply at 155° C. Analysis:— Calcd. for $C_{38}H_{36}O_8$; C, 73.55; H, 5.81%. Found: C, 73.65; H, 5.85%.

Jacobine

The procedure outlined for the isolation of retrorsine from *S. retrorsus*, when applied to *S. jacobaea* with negligible modifications yielded a chloroform extract which on evaporation to a syrup and treatment with alcohol crystallized in a short time. The yield of crystalline product of this grade was only 4.1 gm. from 9.9 kilos of dried material, and only 0.4 gm. was recoverable from the mother liquor.

Jacobine is readily recrystallizable by the addition of methanol to a concentrated chloroform solution. It is only sparingly soluble in alcohol and very sparingly in ether. When slowly crystallized it may be obtained in flat elongated plates with pyramidal terminations. The purest specimen thus far obtained melted at 223-224° C. with vigorous decomposition, some sintering and darkening taking place several degrees lower. Although the pure alkaloid gives no reaction with Ehrlich's reagent the decomposed melt gives an immediate coloration. Analysis:— Calcd. for $C_{18}H_{23}O_6N$; C, 64.86; H, 6.91; N, 4.20%. Found: C, 63.89; H, 7.24; N, 4.35%.

The basic aqueous solution from which the alkaloid had been extracted with chloroform was acidified with acetic acid and boiled to expel chloroform. The charcoaled solution was treated with an aqueous solution of phenylhydrazine

acetate and gently warmed until a dark resin began to separate in small quantity. Charcoal was added and the mixture rapidly filtered with suction. Gentle warming then caused the separation of copper-colored crystalline flakes. The solution was allowed to cool and after 24 hr. the solid was filtered off. (The filtrate on heating for a short time yielded glucosazone in quantity. It was purified by washing with acetone and recrystallizing from hot alcohol by the addition of an equal volume of hot water; m.p. 208° C.) The thoroughly washed phenylhydrazone was dried, dissolved in hot acetone, in which it is quite soluble, and crystallized out by the cautious addition of water. A repetition of the process using alcohol instead of acetone yielded delicate brilliant golden plates, melting sharply at 178° C. The yield of purified product was 70 gm. Admixture with an equal weight of protocatechuic aldehyde phenylhydrazone caused a depression of some 20° C. together with decomposition at 162° C. Analysis:— Calcd. for $C_{13}H_{12}O_2N_2$: C, 68.42; H, 5.26; N, 12.28%; mol. wt. 228. Found: C, 69.14; H, 5.18; N, 12.01; mol. wt. 242 (Rast).

Treatment with dilute or conc. hydrochloric acid with or without formaldehyde leads to extensive resinification. The color is almost certainly inherent in the pure substance, since recrystallization from hot water, in which it is only sparingly soluble, with the aid of charcoal causes no decrease in intensity. The phenylhydrazone is moderately soluble in ether and in chloroform.

Jacobine Methiodide

A solution of jacobine in chloroform quickly deposited the crystalline methiodide when a small amount of methyl iodide was added. The substance is sparingly soluble in alcohol and was recrystallized from water, in which it is moderately soluble even in the cold, by the addition of alcohol. The mixture was filtered off and the crystals were washed with alcohol and then with acetone; colorless flat plates, darkening at 238° and decomposing at 252° C. Analysis:— Calcd. for $C_{18}H_{26}O_6NI$: C, 46.65; H, 5.62; N, 3.02%. Calcd. for $C_{19}H_{26}O_6NI$; C, 48.00; H, 5.47; N, 2.95%. Found: C, 47.41; H, 5.54; N, 3.10%.

Hydrolysis of Jacobine

The procedure adopted in the case of retrorsine when applied to jacobine yielded no sparingly soluble potassium salt. The alcoholic solution was therefore acidified to Congo red with conc. hydrochloric acid and the precipitated potassium chloride filtered off. The filtrate was evaporated to dryness on the steam bath in a rapid current of air and the residue exhaustively extracted with hot ethyl acetate. The insoluble portion yielded retronecine when purified as already described. The hydrochloride, alone or admixed with an authentic specimen, melted sharply at 164° C. and further comparison failed to disclose any differences.

The ethyl acetate extract was evaporated to a syrup and treated with much ether. A small amount of precipitate was obtained which proved to be retronecine hydrochloride. The ether solution on evaporation yielded a syrupy residue which crystallized readily when cautiously treated with petroleum ether. The solid was filtered off, washed with a little ether, in

which it is only sparingly soluble, and recrystallized from much boiling ether.

Jaconecic acid thus obtained crystallizes in stellate aggregates of very fine needles, melting sharply at 178-179° C. Admixed with retronecic acid the mixture sinters at 152° C. and melts completely at 166° C. Analysis:— Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.90%. Found: C, 52.30; H, 7.03%.

Examination of S. aureus for Alkaloids

Dried *S. aureus* (2.25 kilos) obtained from Eimer and Amend of New York was ground to a fine powder and thoroughly extracted with methanol in a Soxhlet extractor. The extract was treated as in the case of *S. retrorsus*. The chloroform extract after removal of non-basic material on evaporation yielded less than 0.5 gm. of a pale yellow resin which could not be obtained crystalline. That a small amount of an alkaloid is present was indicated by the fact that treatment with methyl iodide yielded a trace of a water soluble substance which gave several isolated crystals when the solvent was removed and the residue left in contact with a little methanol. The quantity obtained, however, was insufficient for isolation in a state of purity.

It is therefore not justifiable to state with certainty that an alkaloid is present.

References

1. CUSHNY, A. R. Proc. Roy. Soc. Lond. B. 84: 188-190. 1912.
2. DRAKE, N. L. and BRONITSKY, J. J. Am. Chem. Soc. 52: 3715-3720. 1930.
3. GRANDVAL, A. and LAJOUX, H. Compt. rend. 120: 1120-1123. 1895.
4. SHIMOYAMA, Y. Apoth. Ztg. 7: 453-454. 1892.
5. WATT, H. E. J. Chem. Soc. 95: 466-477. 1909.
6. WEGSCHEIDER, R. Monatsh. 17: 245-252. 1896.

SPONTANEOUS SELF-FERTILIZATION IN RELATION TO SEED PRODUCTION IN SWEET CLOVER (MELILOTUS)[1]

By L. E. Kirk[2] and T. M. Stevenson[3]

Abstract

The chief determining factor in spontaneous self-fertilization in white sweet clover (*Melilotus alba*) appears to be the distribution of pollen within the unopened flowers. This in turn depends on (1) length of stamens, (2) length of style, (3) amount of pollen, and (4) size of cavity within the upper part of the keel. When the flower structure and quantity of pollen is such as to insure the deposition of pollen grains on the stigma before the blossom is likely to be disturbed by insects, the plant will be normally self-fertilized. In the yellow flowered species of sweet clover (*Melilotus officinalis*) spontaneous self-fertilization is effectively prevented, except in certain varieties, by a characteristic of the stigma which makes it unreceptive. Scarification of the stigmatic surface appears to be necessary before fertilization can take place. Variation occurs also, as in *M. alba*, in the length of stamens and style, size of keel cavity, and the amount of pollen.

There is a high correlation in *M. alba* between the percentage of flowers which are naturally self-pollinated and the percentage of flowers which produce pods when the plants are caged to exclude insects. Selection of plants which are normally self-fertilized can be made by examining the early flowers, thus obviating the necessity of bagging or caging plants which do not possess this character.

A strain of *M. alba* which is normally self-fertilized was found to produce almost twice as much seed as another strain which is normally cross-pollinated under comparable conditions in the field.

In a recent article, the writers (1) described certain characteristics of flower structure and behavior in *Melilotus alba* and *Melilotus officinalis* and their relation to the degree of self-fertilization in these species. The results of experiments which were submitted were based on a laboratory study of plants grown in the greenhouse. During the summer season of 1931 an extensive survey was made of a large amount of plant material in the sweet clover breeding nursery for the purpose of finding whether the facts previously ascertained could be verified under field conditions and also to what extent seed production might be affected in actual practice. The results of experiments with plants in the field seem to the writers of sufficient interest to warrant another report of a supplementary nature which may best be read in conjunction with the paper cited above.

These experiments included several varieties of both *M. alba* and *M. officinalis*, some of which were not examined previously, as well as a number of inbred lines of *M. alba*. The same methods were followed as described in the first article. Only flowers with petals unopened or only partially expanded, and which had not been visited by bees, were used. Those which have been visited by honey bees are easily detected because the edges of the keel, when once forced apart, remain partially opened, whereas they are closely pressed together in flowers which have not been disturbed. The flowers were treated

[1] *Manuscript received November 7, 1931.*
Contribution from the Field Husbandry Department, University of Saskatchewan, Saskatoon, Saskatchewan.

[2] *Dominion Agrostologist, Division of Forage Plants, Experimental Farm, Ottawa, Ont. Formerly Professor of Field Husbandry, University of Saskatchewan.*

[3] *Research Assistant, University of Saskatchewan.*

with alcohol and cedar clearing oil, as previously described, to make them transparent and excellent results were always secured with both the white and yellow flowered species providing examination was made at the proper time. If examination is delayed for some time after the flowers become transparent they take on a decidedly abnormal appearance.

Flower Structure and Self-fertilization

Seven factors which appeared to influence self-fertilization were described in the previous article. These were (1) length of stamens, (2) amount of pollen, (3) stage of flower development when pollen is liberated from the anther sacs, (4) receptivity of the stigma, (5) distribution of free pollen within the flower, (6) size of cavity in the upper part of the keel, (7) condition of the pollen. Recent observations justify certain modifications in this list and enable us to obtain a better idea of the different factors as to their probable relative importance.

The relative position of anthers and stigma was thought to depend almost entirely on the length of the filaments. A more extensive examination of varieties and inbred lines has revealed a marked variation also in "length of style". Certain of the inbred lines showed very pronounced differences in this character. Some idea of the comparative lengths of styles may be obtained by observing in Fig. 1 the position of the stigma in relation to the highest point of the keel in the two flowers. The difference is not due to length of keel because it was found by working with a very large number of flowers from different plants in the two selfed lines represented, that the one with the shorter style also had the shorter keel. This would tend to minimize rather than accentuate the apparent difference.

The fact seems to be that both filaments and style are subject to variation in length and it is not always an easy matter to decide which one determines the position of the anthers in relation to the stigma. In many cases it is probable that the length of both are involved at the same time.

With respect to the stage of flower development when pollen is liberated from the anthers it may be said that even less variation was found in the field material than in the greenhouse material. Not a single case was found in which the pollen was not normally liberated from the anthers before the flowers were fully opened. In fact, almost all of the flowers examined contained some anthers which had dehisced before the petals were even partially expanded.

With respect to the germination of pollen grains within the unopened flower which was reported in the earlier paper, it must be said that this character did not show up under field conditions. In the greenhouse studies, germination of pollen within the flower was found to occur to a marked extent in the variety "Redfield Yellow" and also in one plant of *M. alba*. It was not observed in a single one of the numerous plants which were grown in the field including plants of the Redfield Yellow variety. It is difficult, but not illogical, to consider the divergent results as being due to different environmental conditions because, in the same greenhouse, only two out of several varieties and strains exhibited this character. There is still the possibility that inherent differences

in plant material were responsible in view of the fact that observations in the greenhouse were made on a relatively small number of plants, and these special types may not have been encountered in the field.

The chief determining factor in spontaneous self-fertilization in *M. alba* appears to be the distribution of pollen within the unopened flower, since there is a high correlation between the number of flowers which have pollen on the stigma and the degree of actual seed setting. This in turn is determined by four major factors as follows: 1. Length of stamens. 2. Length of style. 3. Amount of pollen. 4. Size of keel cavity. To these a fifth may be added, namely, "receptivity of the stigma", which however appears to be important only in certain types of *M. officinalis*. Table I gives the results of observations in several selfed lines of *M. alba* and shows the combinations of factors which resulted in various percentages of self-pollinated flowers together with the actual percentage of seed setting when the plants were protected from the visitation of insects.

TABLE I

DIFFERENT COMBINATIONS OF FACTORS IN SELFED LINES OF *M. alba* AS THEY AFFECT POLLINATION AND SEED SETTING OF PROTECTED PLANTS

Selfed line	Factors affecting distribution of pollen				Percentage of flowers having pollen on the stigma	Percentage of flowers having produced pods
	Length of stamens	Length of style	Amount of pollen	Size of cavity in upper part of keel		
1	Long	Intermediate	Abundant	Large	100	95.50
2	Intermediate	Short	Scarce	Intermediate	86	80.00
3	Short	Intermediate	Abundant	Intermediate	80	70.60
4	Short	Long	Abundant	Small	55	55.80
5	Intermediate	Long	Intermediate	Small	65	58.80
6	Short	Intermediate	Intermediate	Large	20	18.70
7	Long	Long	Scarce	Intermediate	20	18.00
8	Intermediate	Intermediate	Intermediate	Intermediate	14	10.00
9	Short	Intermediate	Scarce	Small	14	12.00
10	Intermediate	Long	Scarce	Intermediate	3	3.00
11	Intermediate	Long	Very scarce	Intermediate	0	0.00

It is evident from Table I that there was a high correlation between the number of flowers which received pollen on the stigma and the number which produced pods. Line 1 may be considered as 100% self-fertilized, since the examination of more than 100 flowers on each of 34 plants of this strain did not reveal a single blossom that had not been pollinated before being disturbed by insects. Actual counts showed that 95.5% of the flowers produced pods under closely woven cotton cages in the field nursery.

In the process of breeding by selection within self-fertilized lines it may be an important matter to determine before caging, which plants are naturally self-pollinated. This can be done quite easily by examining the first blossoms which appear and observing the relative number of undisturbed flowers which have pollen grains deposited on the stigma. If it is the object to secure lines only which are spontaneously self-fertilized, this method of procedure will obviate the necessity of working with large numbers of unsatisfactory plants.

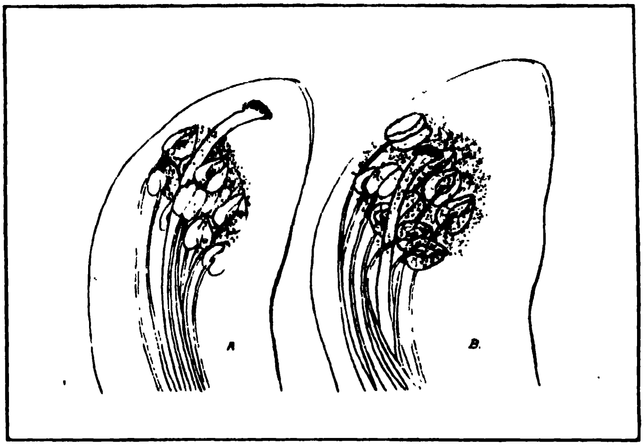


FIG. 1. *Typical flowers from two selfed lines of M. alba showing variation in length of style.*

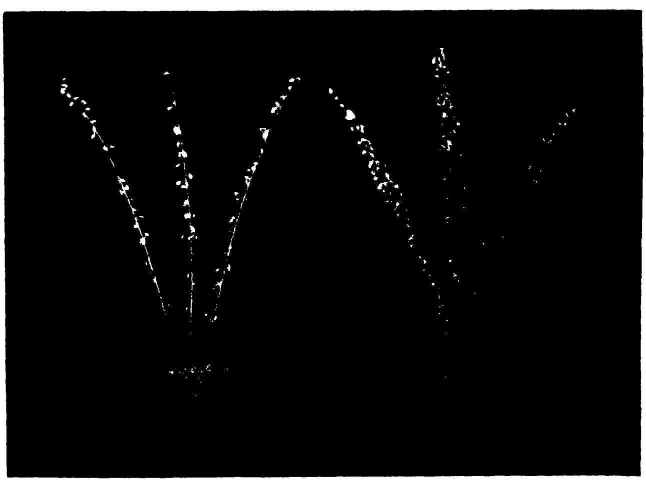


FIG. 2. *Typical seed setting in two selfed lines of M. alba under field conditions showing effect of spontaneous self-fertilization. Left, a normally cross-pollinated strain. Right, a normally self-pollinated strain.*

Self-fertilization in Relation to Seed Setting in M. alba

The value of spontaneously self-fertilized strains of sweet clover is very obvious from the standpoint of maintaining the purity of varieties which possess valuable qualities. This would be a consideration in the case of strains which were bred for resistance to disease. In this connection the question arises also whether strains which are naturally self-pollinated may be expected to produce more seed than others which are naturally insect-pollinated when both are grown under the same conditions and exposed equally to the visitation of insects.

Inbred lines of *M. alba* in the field nursery, which were normally self-fertilized in varying degrees, provided excellent material for estimating the effect of natural self-pollination on the amount of seed produced under ordinary field conditions. For this purpose, five selfed lines were chosen differing widely in the percentage of undisturbed flowers which had been self-pollinated. About 25 plants were examined in each line, and counts made of both flowers and pods on 10 normal racemes from each plant. The number of flowers on each raceme can be determined from the points of attachment on the axis. These are clearly visible with a low power lens. The selfed lines were grown close to one another in the nursery and all were exposed equally to the visitation of honey bees, which were present in large numbers, a large apiary being located nearby.

In the case of plants in the same selfed lines, from which insects were excluded by means of large cotton cages, the determinations on seed setting were made in the same manner as for open-pollinated plants.

Table II gives the percentage of normally self-pollinated flowers in each strain and the percentage of flowers which produced pods when the plants were protected from, and exposed to, the visitation of insects.

TABLE II

RELATIVE SEED PRODUCTION IN SELFED LINES OF *M. alba* WHICH DIFFERED IN THE PERCENTAGE OF FLOWERS THAT PRODUCED SEED NORMALLY BY SELF-FERTILIZATION

Strain number	Percentage of flowers that had pollen on the stigma	Percentage of flowers that produced pods	
		Protected plants (self-pollinated)	Unprotected plants (open-pollinated)
1	100	95 5	99.1
3	80	70 6	73.9
4	55	55 8	68 1
5	65	58 8	74 6
11	0	0	54.1

In Table II, strain 1 represents a selfed line which is completely self-pollinated under all conditions and strain 11, a selfed line which will not normally produce seed unless the flowers have been artificially manipulated or pollinated by insects. It is interesting to observe that strain 1 produced almost twice as many pods per hundred flowers as did strain 11 under conditions favorable for open pollination. Furthermore the amount of seed setting appears to have

been considerably below the optimum in the other strains which were only partially self-fertilized.

The evidence submitted seems to show that strains which are spontaneously self-fertilized may be expected to produce considerably more seed than those which are partially or wholly dependent on insect pollination. This is what one would expect since it was evident from an examination of the older flowers that the bees did not work on every blossom, nor was there pollen on the stigma of every flower which had been visited.

Fig. 2 shows the seed setting on typical racemes at maturity from strain 11 (left) which is normally cross-pollinated, and strain 1 (right) which is normally self-pollinated.

Acknowledgment

The authors express their thanks to Mr. Frank Rose, Field Assistant, for his valuable services in the field and laboratory.

Reference

1. Kirk, L. E. and Stevenson, T. M. Factors which influence spontaneous self-fertilization in sweet clover (Melilotus). Can. J. Research, 5: 313-326. 1931.

THE TYPES OF OSMOPHILIC YEASTS FOUND IN NORMAL HONEY AND THEIR RELATION TO FERMENTATION[1]

BY A. G. LOCHHEAD[2] AND LEONE FARRELL[3]

Abstract

A study of the predominant sugar-tolerant yeasts infecting 163 samples of normal Canadian honey led to the recognition of eight different species, comprising the genera *Zygosaccharomyces*, *Schizosaccharomyces* and *Torula*. The frequency of their occurrence varied greatly, one type, *Z. richteri*, being by far the most commonly encountered. The yeast predominating originally is not necessarily the most abundant type after fermentation. Analysis of samples fermenting within 14 months showed species of *Zygosaccharomyces* only to be most abundant, while *Z. richteri*, in addition to being the predominant type infecting a large majority of samples, was able, even in certain cases where it was originally outnumbered, to develop and apparently assume the leading role in fermentation.

Introduction

In a previous investigation reported from this laboratory (8), a study was made of the infection of normal honey by sugar-tolerant yeasts. An examination of 191 samples, representing all parts of Canada, showed the presence of yeasts in all cases though the amount of infection, as indicated by yeast counts, varied widely. It was found, moreover, that the tendency to ferment increased with increasing yeast infection, which latter was believed to be a factor directly affecting fermentation.

To note whether the types of osmophilic yeasts, as distinct from the numbers present, are of importance in causing fermentation the present study was undertaken. The yeast types occurring most abundantly in the original samples were first determined. The honeys were then placed in storage and examined for fermentation. From those which fermented within a 14-month storage period the most abundant yeast types were again isolated and studied.

Experimental

In the study referred to above, yeast counts were made by the dilution method, the medium employed consisting of two parts by weight of honey diluted by the addition of one part of a nutrient solution containing, per litre; peptone, 1.0 gm.; K_2HPO_4, 1.0 gm.; $MgSO_4$, 0.5 gm.; ammonium tartrate, 0.5 gm.; NaCl, 0.1 gm.; and $CaCl_2$, 0.1 gm. For the present study, the tubes which showed fermentation with the greatest dilutions of honey were used and from them yeasts were isolated by plating on 60% honey agar. Plates were incubated at 28° C. and isolations made from all colonies which exhibited any macroscopic differences. The cultures isolated were purified by replating three times on the same medium preparatory to the detailed morphological

[1] *Manuscript received October 8, 1931.*
Contribution from the laboratories of the Division of Bacteriology, Dominion Experimental Farms, Ottawa, with the co-operation of the National Research Council of Canada. The authors are indebted to Mr. C. B. Gooderham, Dominion Apiarist and staff of the Bee Division, Dominion Experimental Farms, for obtaining the samples and examining them for fermentation during storage.
[2] *Dominion Agricultural Bacteriologist, Department of Agriculture.*
[3] *Chemist, Associate Committee on Honey Research, National Research Council of Canada.*

and physiological examination. In this way 201 cultures were obtained from 163 different samples of normal honey.

For comparison and identification of the cultures isolated, transfers were made on 50% honey agar slants, on 15% honey agar flasks for giant colony formation, and into dextrose, saccharose and maltose broth prepared by adding the sugar in 10% concentration to a basic solution of 0.5% yeast extract broth. Microscopic and macroscopic examination of the growth on solid media with a comparison of the fermentative characteristics permitted an elimination of many cultures. After this preliminary survey all remaining cultures were studied in greater detail according to the following plan:

(a) Microscopic observation on solid and liquid media.
(b) Growth on honey agar slants, containing 15% and 70% honey respectively.
(c) Growth in honey broth of 15% and 70% honey.
(d) Giant colony formation on 15% honey agar.
(e) Growth on carrot, potato, in milk and in gelatine media containing 15% and 70% honey respectively.
(f) Fermentation tests with the following: arabinose, xylose, dextrose, levulose, mannose, galactose, saccharose, maltose, lactose, raffinose, dextrin and mannite.
(g) Comparison of growth at 37° C. on honey agar of high and low concentration (70% and 15%).

As a result of these tests the cultures were finally reduced to eight types, sufficiently distinct as to morphology and cultural characteristics as to warrant being considered different species. In addition, two types, closely related to two of the species, were classed as subspecies. Of the eight types six were classified as *Zygosaccharomyces*, one as *Schizosaccharomyces* and one as *Torula*.

Duplicate samples of the honeys from which the above isolations were made were held in storage at room temperatures and observed for fermentation. Samples which were found to have fermented within a 14-month period were analyzed for total yeast count by the dilution method previously described (8). To determine the yeast types occurring most abundantly, isolations were made as before from the tubes showing fermentation with the highest dilution of honey. In all, 30 samples of fermented honey were examined and the cultures isolated were subjected to the same process of comparison as previously described for those from normal honey. In this manner, four species, with two related subspecies of sugar-tolerant yeasts, all of the genus *Zygosaccharomyces*, were distinguished.

The frequency with which the various types of sugar-tolerant yeasts were found as the predominating organism in normal honeys varied widely. As may be seen from Table I, one type, culture 20, occurred much more frequently than any of the others, some being isolated in but one instance. It will be noted, furthermore, that in the case of but three yeast types, representing a small fraction of the samples, did the honeys in question all remain unfermented. In all cases of fermentation the originally predominant organisms were found to be *Zygosaccharomyces*. The belief that members of this genus are primarily

concerned with fermentation is supported by the results of the examination of the fermented samples. In every instance the predominant yeast type proved to be of the genus *Zygosaccharomyces*.

From Table I it will be observed that in many cases the yeast type predominating originally did not prove to be that most abundant in the honey after fermentation. This leads to the belief that the organism with which a sample of honey may be most heavily infected is not necessarily the causal agent of fermentation. Thus culture 78, which was found as the predominant type in 23 samples, of which 7 fermented, was not isolated in any of these cases after fermentation. On the other hand, yeasts of the culture 20 group,

TABLE I

INCIDENCE OF OSMOPHILIC YEASTS IN NORMAL HONEY AND IN FERMENTED SAMPLES

Culture No.	Classification	Times predominant in normal honey	Samples fermenting within 14 months	% Fermented	Types predominating after fermentation								
					Types in original samples								Other types
					20	78	138	16X	155Y	139	58	11Y	
20	*Zygosaccharomyces*	120	22	18.3	20	0	1	0	0	0	0	0	1
78	*Zygosaccharomyces*	23	7	30.4	6	0	0	0	0	0	0	0	1
138	*Zygosaccharomyces*	16	4	25.0	0	0	2	0	0	0	0	0	2
16X	*Torula*	9	0	0.0	-	-	-	-	-	-	-	-	-
155Y	*Zygosaccharomyces*	8	2	25.0	1	1	0	0	0	0	0	0	0
139	*Zygosaccharomyces*	7	2	28.6	2	0	0	0	0	0	0	0	0
58	*Zygosaccharomyces*	1	0	0.0	-	-	-	-	-	-	-	-	-
11Y	*Schizosaccharomyces*	1	0	0.0	-	-	-	-	-	-	-	-	-

found as the predominant type in 120 samples of which 22 later fermented, was found after fermentation, not only in 20 of these cases, but also in 9 samples in which the originally predominant yeasts were of other types. Culture 20 and related types are considered to be *Zygosaccharomyces richteri*, first isolated from fermented honey (7), but also found as the most common yeast infecting hive nectar (7). It has also been isolated from the nectar of various flowers (7) and from apiary soil (6). Altogether it appears to be the most ubiquitous sugar-tolerant yeast concerned with honey. Not only is it the predominant yeast infecting a large majority of samples, but even in cases where it is outnumbered by other types, it is apparently able to develop and assume the leading role in honey fermentation.

Yeasts Isolated from Normal Honey

Of the eight species isolated from normal honey, five appeared to be identical with yeasts already reported from this laboratory (6, 7), and consequently a detailed description of their morphological and cultural characteristics is not repeated here. Their classification, however, may be briefly indicated.

CULTURE 20 (*Zygosaccharomyces richteri*) (see Fig. 1)

This yeast, which ferments dextrose, levulose, and mannose is considered identical with culture M1, isolated from fermented honey (7), and culture S3B3 found in apiary soil (6) and previously described. Some strains show a

slight tendency to ferment saccharose. On 15% honey agar the giant colony is normally smooth at first with concentric and radial markings, the surface becoming later covered with a dotted growth. The form of growth, however, is less characteristic than in the case of most of the other species found.

CULTURE 78 (*Zygosaccharomyces nussbaumeri*) (see Fig. 2)

This yeast, which shows a characteristically raised and folded growth on such media as 15% honey agar and carrot, ferments dextrose, levulose, mannose, saccharose and maltose. It has been previously described as culture J7 from fermented honey (7) and culture S3B6 from apiary soil (6).

CULTURE 16X (*Torula sp.*) (see Fig. 3)

Forming a very characteristic giant colony on 15% honey agar, this yeast is readily distinguished. Fermenting dextrose, levulose, mannose, saccharose and raffinose, it is identical with culture S3C3, isolated from apiary soil and previously described (6). From one sample a culture (11X), considered a subspecies of 16X, was isolated. While agreeing with the latter in other respects, it was unable to cause fermentation of saccharose and raffinose. Otherwise the description will suffice.

CULTURE 155Y (*Zygosaccharomyces sp.*) (see Fig. 4)

The growth of this yeast on such media as 15% honey agar, carrot, potato is usually slow and scanty. On 15% honey agar colonies are characteristically small and raised. Dextrose, levulose and mannose are fermented. With this yeast fermentation is much more vigorous in 70% than in 15% honey broth. This type is regarded as the same as N4, from floral nectar (7), and culture S3B2, isolated from apiary soil and previously described (6).

CULTURE 139 (*Zygosaccharomyces sp.*) (see Fig. 5)

This yeast produces a characteristic growth on 15% honey agar. The surface of the colony, at first smooth, becomes later heaped up into coarse folds particularly in the central portion, facilitating recognition. Dextrose, levulose, mannose, saccharose and maltose are fermented. This yeast is identical with culture S3B11, isolated frequently from apiary soil (6).

In addition to the five species indicated above, three species were isolated from normal honey, the descriptions of which may be given in greater detail.

CULTURE 138 (*Zygosaccharomyces sp.*) (see Fig. 6)

In young cultures on 15% honey agar, cells are ellipsoidal to round, occurring singly, in pairs and in small groups and chains of adherent cells. The cells vary in length from 3 to 7 μ and in width from 2 to 4.5 μ, the average size being 4.5 by 3 μ. In old cultures on 70% honey agar there is some tendency to form hyphae of elongated cells. Reproduces asexually by budding. Spore formation, resulting from isogamic copulation may be observed on such media as honey agar, honey gelatine and honey broth. Ascospores were observed regularly to be two in number, of a diameter of approximately 3.0 μ.

Honey agar 15%.—Abundant filiform growth, light cream colored, with surface smooth at first but usually somewhat folded in older cultures; edge finely lobate; lustre dull; soft cheesy consistency.

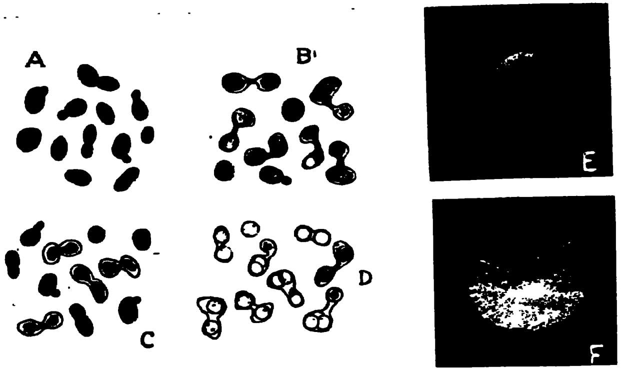


FIG. 1. CULTURE 20. *A. Young culture, honey agar 15%, three days, showing budding. B. Ten-week culture, honey agar 70%, showing copulation and ascospore formation. C. Carrot culture, eight days, showing copulation. D. Culture on honey gelatine, nine weeks, showing ascospores. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 164 days.*

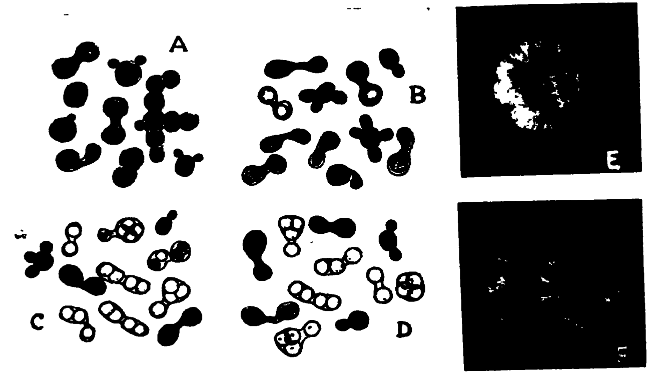


FIG. 2. CULTURE 78. *A. Young culture, honey agar 15%, three days. B. Eight-day culture on carrot, showing copulation and ascospore formation. C. Ten-day culture, honey agar 15%, showing ascospores. D. Culture on honey agar 70%, 10 weeks, showing ascospore formation. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 110 days.*

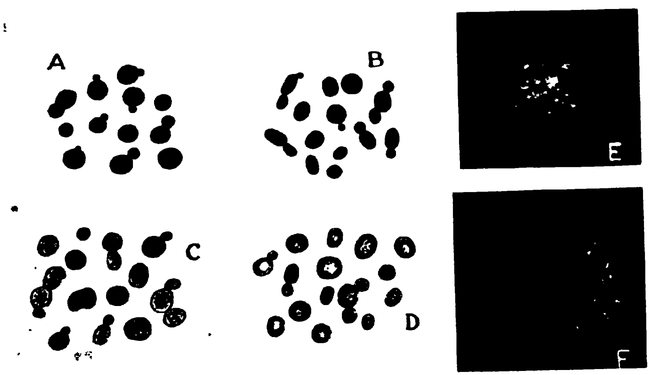


FIG. 3. CULTURE 16X. *A. Young culture, honey agar 15%, three days, mostly round cells, showing budding. B. Potato culture, seven days, showing ellipsoidal cells. C. Nine-week culture, honey agar 70%. D. Culture on carrot, nine weeks, showing thick-walled cells. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 110 days.*

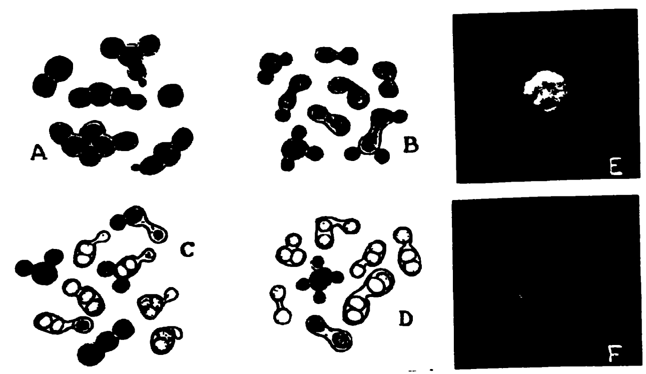


FIG. 4. CULTURE 155Y. *A. Young culture, honey agar 15%, three days showing budding. B. Eight-day culture, honey agar 15%, showing copulation C. Carrot culture, nine weeks, showing ascospores. D. Nine-week culture, honey agar 70%, showing ascospores. E. Giant colony, honey agar 15%, 34 days F. Giant colony, honey agar 15%, 146 days.*

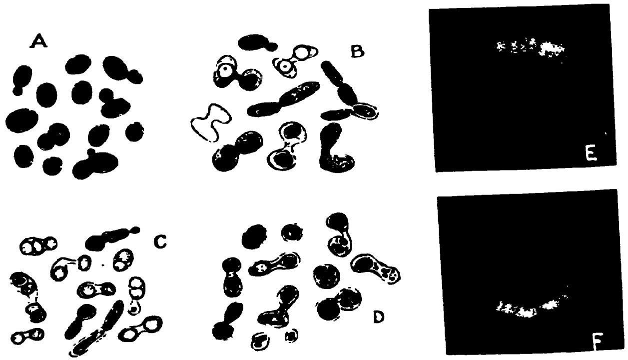


FIG. 5. CULTURE 139. *A. Young culture, honey agar 15%, three days, vegetative cells. B. Four-week carrot culture, showing copulation and ascospore formation. C. Nine-week carrot culture, showing ascospores, also elongated cells. D. Culture on honey gelatine 15%, showing ascospore formation by copulation, also apparently by parthenogenesis. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 59 days.*

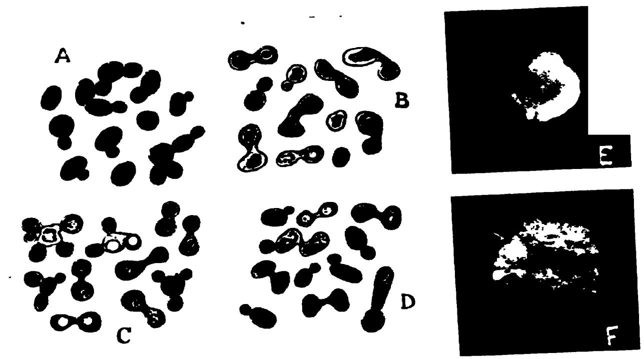


FIG. 6. CULTURE 138. *A. Young culture, honey agar 15%, three days, vegetative cells. B. Culture on honey gelatine 15%, eleven weeks, showing copulation. C. Surface ring growth, honey broth 15%, eleven weeks, showing copulation and ascospore formation. D. Eleven-week culture, honey agar 70%, showing ascospore formation, also elongated cells. E. Giant colony, honey agar 15%, 22 days. F. Giant colony, honey agar 15%, 74 days.*

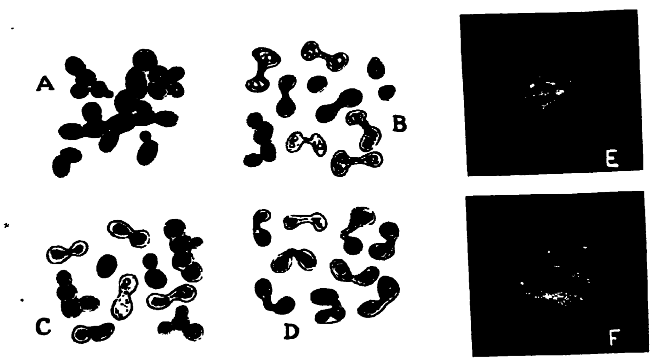


FIG. 7. CULTURE 58. *A. Young culture, honey agar 15%, four days, vegetative cells. B. Ten-week culture, honey agar 70%, showing copulation. C. Carrot culture, 15 days, showing copulation, also thick-walled vegetative cells. D. Four-week culture on potato, showing copulation and ascospore formation. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 59 days.*

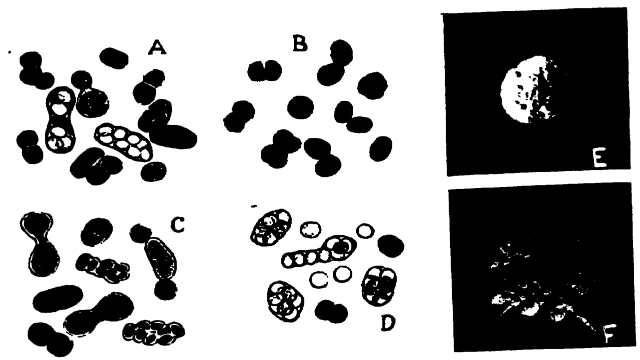


FIG. 8. CULTURE 11Y. *A. Young culture, honey agar 15%, showing vegetative cells, also ascospores. B. Surface ring growth, honey broth 70%, showing multiplication by fission. C. Seven-day culture, honey agar 70%, showing copulation. D. Eight-day culture, honey agar 15%, showing ascospores. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 59 days.*

Honey agar 70%.—Moderate filiform growth, light brown in color, smooth except where surface may be broken by gas; somewhat glistening; consistency slightly viscous.

Honey broth 15%.—Active alcoholic fermentation; surface ring growth; abundant flocculent sediment with liquid finally becoming clear.

Honey broth 70%.—Active fermentation; brownish surface ring growth.

Giant colony, honey agar 15%.—General form of colony round, with margin very irregular and lobate; raised with irregular broken surface with concentric and radial markings; cream to flesh colored, tending to darken with age; dull lustre and soft cheesy consistency.

Carrot.—Growth abundant, somewhat spreading and raised, cream colored; surface contoured and smooth, at first glistening, though less so in older cultures; soft, butyrous consistency; medium unchanged.

Potato.—Moderate cream colored growth, somewhat raised, with contoured surface; slightly glistening; butyrous to soft cheesy consistency; medium unchanged.

Milk.—After 10 weeks, no visible change.

Gelatine.—No liquefaction observed after 10 weeks in honey gelatine 15% and 70%.

Growth at 37° C.—Growth on 70% honey agar, none on 15%.

Fermentations.—Dextrose, levulose, mannose and maltose are fermented with acid and gas formation. (A closely related type, culture 9, while similar in all other respects, did not ferment maltose and was classed as a subspecies.) No fermentation was observed with arabinose, xylose, galactose, saccharose, lactose, raffinose, dextrin and mannite.

The characteristics of this culture appear to coincide well with those of the yeasts of Group I of Fabian and Quinet (2), and is accordingly classed with this group. These workers regarded their type as *Zygosaccharomyces japonicus* Saito. With our culture as with theirs, however, film formation, a characteristic of *Z. japonicus*, was not observed. We are inclined to regard our yeast as related to *Zygosaccharomyces priorianus* Klöcker, isolated from the bodies of bees (4) and showing the same fermentative properties. Osmophilic yeasts, closely related to *Z. priorianus* and capable of causing fermentation in the concentrated wine must of "Trockenbeerenauslesen" have been isolated recently by Kroemer and Krumbholz (5).

CULTURE 58 (*Zygosaccharomyces sp.*) (see Fig. 7)

Young cultures on honey agar show mostly ellipsoidal cells though occasionally cylindrical types may be noted and a few round forms. Cells occur generally in groups formed by budding, with single cells and pairs being less frequently seen. There is considerable variation in size, cells ranging from 2.5 to 8 μ long by 2 to 5 μ in width. The majority of the cells are 3.5 to 5 μ long by 3 to 4 μ wide. In 70% honey broth, the cells are somewhat smaller. Old cultures on 70% honey agar show rather thick-walled cells, round to elongated with the occasional appearance of rudimentary mycelium. Reproduces asexually by budding. Zygospores are formed as a result of isogamic

copulation on such media as potato and 70% honey agar, ascospores appearing most frequently to the number of two, though one to three in the ascus were sometimes noted.

Honey agar 15%.—Moderate filiform growth, cream to fawn colored, with irregular, lobate edge; slightly raised, with surface glistening at first, but later becoming dull as it assumes a granular appearance; consistency firmly butyrous.

Honey agar 70%.—Moderate light brown, filiform growth, slightly raised, with smooth surface, except where broken by gas; margin finely wrinkled; slightly viscous consistency.

Honey broth 15%.—Active fermentation; light brown surface ring growth; abundant, coarsely flocculent sediment with liquid clearing.

Honey broth 70%.—Active fermentation; brownish surface ring growth.

Giant colony, honey agar 15%.—Colony rather small, irregular in shape with lobate edge; raised with irregular surface which assumes a characteristic, finely granular appearance; dark cream colored with dull lustre; consistency butyrous to cheesy.

Carrot.—Abundant, cream colored growth, slightly raised, with smooth, contoured surface; at first glistening; soft butyrous consistency; medium unchanged.

Potato.—Growth less abundant than on carrot; raised with dull surface; cheesy consistency; medium unchanged.

Milk.—No change observed after 10 weeks.

Gelatine.—Liquefaction of honey gelatine 15%; none observed in 70% after 10 weeks.

Growth at 37° C.—Good growth on honey agar 70%, only slight growth on 15%.

Fermentations.—Dextrose, levulose, mannose and saccharose are fermented. No fermentation observed with arabinose, xylose, galactose, maltose, lactose, raffinose, dextrin and mannite.

This yeast has the same fermentative properties as Group II of Fabian and Quinet (2) and culture E6, previously isolated from fermented honey in this laboratory (7). The above types were regarded as related to *Zygosaccharomyces barkeri* Saccardo-Sydow. Culture 58, however, exhibits both cultural and physiological differences from these types, and is also distinguished from *Z. nadsonii* by its isogamic copulation. It has, therefore, not been identified with other described species.

CULTURE 11Y (*Schizosaccharomyces octosporus*) (see Fig. 8)

From one sample of honey a yeast belonging to the comparatively rare genus *Schizosaccharomyces* was isolated. Members of this genus appear to be mainly tropical yeasts, and hence it was a matter of considerable interest to find a representative in a sample of Canadian honey. The honey in question was produced in the southern interior of British Columbia, in a warm dry district requiring extensive irrigation. It had the lowest moisture content of the samples examined, 15.9%.

In young cultures on honey agar 15%, round and elongated cells are seen,

about 5μ in diameter. Multiplication occurs by transverse division, a partition wall appearing in the middle of the cell. The two daughter cells become round in shape, and may finally separate or remain attached, and in their turn divide again into two cells by the same process of fission. Thus groups of 4 or more cells may be noted, giving an appearance similar to *Sarcina*. Ascospore formation occurs very readily on the media employed. It may occur as the result of a fusion of cells or apparently in elongated cells without copulation. The asci are comparatively large, 5 to 8 μ wide and 12 to 20 μ in length. The spores were found regularly to the number of 8 in the ascus, oval to round and measuring usually 4 to 5 μ long by 3.5 μ wide. Free spores appeared to be round.

Honey agar 15%.—Moderate filiform cream colored growth, soon becoming covered with globular surface outgrowths, light brown in color, and giving a verrucose appearance; lustre dull; consistency rather dry and cheesy.

Honey agar 70%.—Moderate filiform growth, light brown in color; slightly raised with surface becoming irregular, showing numerous ridges and cracks; dull, with butyrous consistency.

Honey broth 15%.—Alcoholic fermentation with gas; slight light brown ring growth round side of tube; abundant, finely divided sediment with liquid becoming clear.

Honey broth 70%.—Active alcoholic fermentation; brownish surface ring growth.

Giant colony, honey agar 15%.—Cream colored convex colony with irregular margin and smooth, rather dull surface on which secondary outgrowths occur, particularly in central portion; these globular outgrowths, darker in shade, finally cover the surface, giving the colony a characteristic verrucose appearance.

Carrot.—Growth slow and scanty, heaped up, giving a beaded appearance; dark cream colored and dull, with soft, cheesy consistency.

Potato.—Growth scanty, raised and beaded along line of inoculation; fawn colored at first, soon becoming chalky; dull, with cheesy consistency.

Milk.—After 10 weeks reaction slightly alkaline, otherwise no change.

Gelatine.—Liquefaction in honey gelatine 15%; none in 70% after 10 weeks.

Growth at 37° C.—None observed on honey agar 15% or 70%.

Fermentations.—Dextrose, levulose and maltose are fermented. No fermentation observed with arabinose, xylose, mannose, galactose, saccharose, lactose, raffinose, dextrin and mannite.

This yeast appears to correspond with *Schizosaccharomyces octosporus* isolated by Beijerinck from dried currants (1), and conforms well with the morphological and fermentative properties of this species as described by him. According to Guilliermond (3) *Sch. octosporus* is able to ferment other sugars than those indicated. Beijerinck, however, in testing a series of carbohydrates, found dextrose, levulose and maltose capable of being assimilated, with very sparse growth with mannite and dextrin. The author indicates good fermentation only in the case of dextrose, levulose and maltose.

Yeasts Isolated from Fermented Samples

Of the four types isolated from the samples after fermentation, three corresponded with yeasts from the original samples (see Table I). The fourth type, while agreeing in most characteristics with culture 20, fermented saccharose and maltose, and a comparison with yeasts previously described inclines us to classify it with Group IV of Fabian and Quinet (2).

References

1. Beijerinck, M. W. Centr. für Bakt. 16: 49-58. 1894.
2. Fabian, F. W. and Quinet, R. I. Mich. Agr. Exp. Sta. Tech. Bull. 92. 1928.
3. Guilliermond, A. The Yeasts (transl. by F. W. Tanner), Wiley. New York. 1920.
4. Klöcker, A. Handbuch der technischen Mykologie (F. Lafar). 2. Aufl. Jena. 4: 141-191. 1907.
5. Kroemer, K. und Krumbholz, G. Arch. für Mikrobiologie. 2: 352-410. 1931.
6 Lochhead, A. G. and Farrell, Leone. Can. J. Research, 3: 51-64. 1930.
7. Lochhead, A. G. and Heron, D. A. Dom. of Can. Dept. Agr. Bull. 116. n.s. 1929.
8. Lochhead, A. G. and McMaster, N. B. Sci. Agr. 11: 351-360. 1931.

EPIDEMICS AMONG SLEDGE DOGS IN THE CANADIAN ARCTIC AND THEIR RELATION TO DISEASE IN THE ARCTIC FOX[1]

By Charles Elton[2]

Abstract

An enquiry was made by the Hudson's Bay Company into the origin and spread of a serious disease resembling epidemic encephalitis of silver foxes which periodically destroys large numbers of sledge dogs in the arctic and subarctic regions of Canada. A similar disease occurs in the arctic fox, and is associated with an important four-year cycle in the numbers of the fox, which may thus form a permanent reservoir for the disease organism, or organisms. General forecasting of this fox cycle is possible, and is dependent on knowledge of the lemming cycle in the arctic, and associated climatic phenomena.

Introduction

During the course of a systematic enquiry into the fluctuations in numbers occurring among Canadian wild animals, carried out on behalf of the Hudson's Bay Company during the last five years, the author became aware of the existence of considerable epidemics among the sledge dogs of the Canadian Arctic, in particular, in the region of Baffin Island, Hudson Strait, and Hudson Bay. At the same time a certain amount of information was collected concerning a very similar disease which breaks out periodically among arctic foxes in the North; the existence of the latter was first pointed out to the writer by Mr. L. Romanet, through Professor William Rowan, and later in personal conversation.

The Hudson Bay Company sent questionnaires to certain of their posts in the North, in order to find out how much was known about the sledge dog and fox diseases, and the Company have allowed the writer to place on record here the results of the enquiry, together with some other research carried out through their organization. The author is indebted to Mr. Charles V. Sale (the late Governor), the Committee of the Company, and to Chief Factor C. H. French, the late Fur Trade Commissioner in Canada, for affording every possible facility during the course of this work.

Investigation revealed the fact that epidemics among sledge dogs are often on a disastrous scale (thus 90% of the dogs at Stupart's Bay post were killed during one visitation), and furthermore that the disease had broken out periodically for a number of years past. There is also some evidence that it has been becoming more frequent in recent years. The great importance economically, both for the white population and for the natives, of this periodic destruction of their chief means of transport in winter needs no stressing; but a study of the whole problem also has a direct bearing upon the fur trade itself, since the white fox epidemics form an important part of the violent cycle in fur production which links the white people and the natives into a common economic interest. Both aspects of the question may also have more serious

[1] *Manuscript received May 22, 1931.*

[2] *Demonstrator, Department of Zoology and Comparative Anatomy, University Museum, Oxford, England.*

relations still to the welfare of the Eskimos, since lack of winter transport or of fur often causes them great hardship.

It would be preferable to present the results of this research in a finished state, but it is apparent that at least several years of co-operative effort on the part of Government, commercial, and scientific bodies, will be required before the exact nature of these epidemics can be ascertained and effective control obtained. There is another aspect too: recent research in Minnesota University carried out by Dr. R. G. Green and others (6, 7, 8, 9), has brought to light a wide-spread disease, known as fox encephalitis, which may at times attack and kill a large percentage of the animals on silver fox farms. This disease resembles in many ways that which occurs in the North among dogs and foxes, and may prove to be the same. It would therefore seem important to bring together the results of these two lines of research at the earliest possible moment. Hence it was thought desirable to present at once the preliminary results of the Hudson's Bay Company's enquiry, in order to focus attention on the problem. The writer is greatly indebted to Dr. S. Hadwen for his constant advice and interest during this investigation, and to Dr. R. G. Green for some valuable suggestions.

Acknowledgment is also made of the value of the observations supplied by various officials in the Hudson's Bay Company and other persons, in particular the help given by Mr. George Binney, who made certain special enquiries about arctic fox cycles, when he was travelling round Hudson Bay, and by Mr. C. S. Townsend, late Development Manager of the Company. Their co-operation and help have made it possible to write this paper: wherever names are known the information is acknowledged in the text.

Epidemics Among Sledge Dogs

Questionnaires were framed as follows and sent out to a number of the Company's fur posts in the North:

"A fatal epidemic disease has become common among sledge-dogs in the North, and is believed to depend in some way upon similar epidemics among white foxes. The disease is shown by strange behavior on the part of the dogs, which foam at the mouth and run about in a peculiar way, finally becoming weaker and weaker until they die. It is extremely important that the nature of this epidemic should be discovered, in order that its spread may be checked. You are asked to supply any information you possess in answer to the questions given below:

"1. Has any epidemic of this sort occurred among dogs at your Post? When did it first occur?

"2. What were the symptoms of the disease?

"3. In what month of the year did it occur?

"4. Have you ever observed any epidemic disease among *White Foxes?*

"5. In what month and year did you observe it?

"6. What were the symptoms of the disease?

"7. Have you ever observed any connection between the disease in the *dogs* and that in the *White Foxes?*"

The questionnaire was based chiefly on descriptions of the disease given to the writer by Mr. W. O. Douglas who had for some years been manager of the post at Baker Lake. A summary of the replies received to these questions is given below, dealing in this section with the first three. In a few cases data from other sources are included. The places mentioned are shown on the map in Fig. 1.

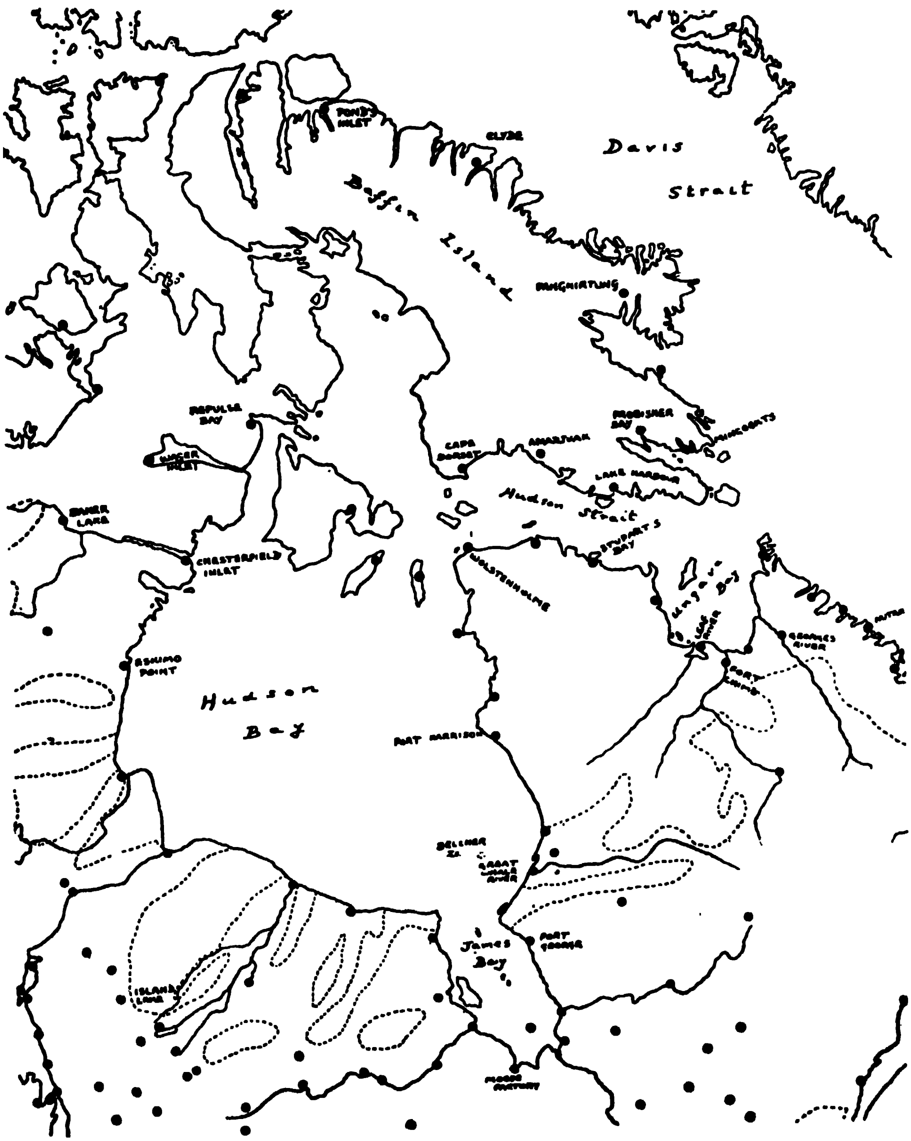


FIG. 1. *Map of Arctic Canada (based on the official map of the Hudson's Bay Company, by permission). Black dots are Hudson's Bay Company posts and outposts (except in the case of Mingookts). The map contains only names of posts mentioned in the text. Approximate tree-limit is shown by dotted line. Scale, about 270 miles to one inch.*

George's River Post

Epidemic killed many dogs about 1921 and again in 1928, March and April. Symptoms: frothing at mouth, partial paralysis and viciousness. (From Alexander Smith, summer, 1929.)

Fort Chimo Post

Epidemics twice during the last seven years. Mostly in spring and summer, but not restricted to any one month. Symptoms vary: (1) Slight foaming at mouth, apparent giddiness, snapping at any noise close by as if they were blind, vomiting, and except in rare cases, dying in the course of a few days. (2) Loss of power of hind quarters, continual snapping at all approaching, whether man or beast, and very savage, frequent recoveries, but liable to recurrence or; (3) savage at any restraint, absolutely wild, liable to attack people, run at a tree, house, or any large object that crosses their vision, or a mad desire to gallop. These three sets of symptoms may be separate, or intermingled to a varying extent. (From John Blackhall, summer, 1929.)

Leaf River Post

Winter 1928-29; an epidemic killed off most of the Eskimos' dogs, every team being reduced to one or two dogs. Broke out in March and continued until June. Symptoms: first, the dogs became very weak and bad tempered. As the disease advanced some dogs lost power over their hind quarters, others of their fore legs. All foamed at the mouth and became very savage. The sickness was not incurable as a few of the Post dogs, which were very sick, recovered after administering sulphur and lard. (From Robert Skinner, summer, 1929.)

Stupart's Bay Post

Broke out in October 1927, lasting until March 1928, killing 90% of the dogs at this Post. Symptoms: foaming at mouth, weakness of legs, dogs eventually falling down and dying. (From W. T. Watt, summer, 1929.)

Wolstenholme Post

Epidemic among the Post dogs about the middle of March 1928. Symptoms: (1) foaming at the mouth and running round in a dazed manner; (2) the dogs simply pined away; diarrhoea appeared to be the chief symptom, and in some cases small amounts of froth mingled with blood were passed. There were these two distinct types of disease. Dogs which suffered from the first only recovered and are still working. None recovered from the second. *Four or five half-breed dogs and a St. Bernard at the Post did not contract the disease.* The epidemic was at its worst amongst the young dogs, most of the recoveries being in the cases of old dogs.

A further note written in May 1930 stated that the mad type of epidemic had occurred in three dogs in the spring of 1930. Symptoms: first stage, lasting about two days, excited behavior, with tongue lolling, panting as if overheated, giving a short sharp bark or "yap" occasionally, also snapping at anything that comes near them. They then begin to "chew" and foam at the mouth, and have a peculiar glassy stare and do not seem to recognize anyone; if not tied up they will sometimes run around in a staggering manner, fall

down as if in a fit and not recover, but mostly they have to be shot. He states that any dog bitten by one with this disease usually shows the same symptoms in about eight days. (From F. Melton, summer, 1929.)

Lake Harbor Post

Epidemics in dogs a few years previous to 1926 (Post Manager's information, through Mr. G. Milling). He had noticed the hanging jaw symptom (see below).

Frobisher Bay Post

Two epidemics during the past few years. First began in November 1924, when practically all the dogs died. Symptoms: as described in the questionnaire, strange behavior, foaming at mouth, running in a peculiar way, finally becoming weaker and weaker until they died.

The second epidemic began in June 1927 and broke out again in September 1927. Symptoms slightly different: dogs became very fierce at first, and went pugnaciously among the other dogs. Small dogs in this condition easily overcame the strongest dogs, whereas before the epidemic they would cringe with their tails between their legs even at a growl from the big dogs. This stage lasted several days, after which the dogs began to foam at the mouth and go about with the mouth wide open, apparently having the jaw locked so that they could not close the mouth. The dog afterwards moved about peacefully and slowly until he died. The course of the disease lasted about three weeks. The epidemic first appeared among the Sabellum Company's dogs at Mingetook (apparently the same as Mingookts in Frobisher Bay) in April 1927. *None of the Post mongrels were affected.* (From James Bell, summer, 1929.)

Clyde Post

Some of the older natives claim they have seen a fatal disease in dogs, but a long time ago. (From J. Smith, March 3, 1930).

Pond's Inlet Post

Last epidemic in spring 1924. "During that time I believe the disease was prevalent amongst all dogs in Baffin Land". Symptoms as in questionnaire: increasing weakness leading to death. One dog became violent and chased several Eskimos around until it was finally shot. Natives remembered severe epidemic about fifteen years ago, which killed nearly all the dogs in the country. (From F. G. Troup, May 31, 1930.)

Repulse Bay Post

"This epidemic occurs at this Post regularly each spring, also often in the fall, and this year it has attacked and destroyed a number of pups during the winter. Symptoms: green pus in eyes, nose choked with same pus, partial paralysis of hind quarters. Fatal in nine cases out of ten. (From McHardy, April 11, 1930.)

Wager Inlet Post

A few pups died September 1929 (symptoms not recorded owing to absence of manager). No epidemic since. In 1928-29 nine full-grown dogs died with symptoms similar to those recorded below for Chesterfield Post. (From the Post Manager, summer, 1930.)

Chesterfield Post

Epidemic disease during 1925-1928, mostly during August to September, usually stopping as soon as the cold weather set in. Comparatively few die in winter, mostly younger dogs. Symptoms: paralysis of hind-quarters, generally howling as if in great pain, frothing at mouth, eyes glassy with yellow matter deposited around them, noses stopped up with similar substance. (From the same Post Manager mentioned under Wager Inlet Post through Mr. Hugh Conn, the Company's General Inspector; see also under Baker Lake Post and Moose Factory Post.)

Baker Lake Post

Epidemic broke out in September 1929, lasting until end of October. Five dogs died. Symptoms: foaming at mouth, complete loss of appetite, eyes dull and diffused, partial paralysis of hind quarters, would stagger round in circles and keep falling down. Later on, a chronic discharge of pus from the nose and eyes, and total paralysis of the hind quarters. This usually continued for about a week when they finally died from weakness. "In the many instances I have witnessed an outbreak of this disease, I have never known a dog to attack any person or other dog."

In a personal interview with Mr. W. O. Douglas in London, the following information was obtained. He first came to Baker Lake in 1916. At this time, he stated, the sledge dogs had epidemics periodically about every fourth year, corresponding with a similar cycle in white fox epidemics. Now (1929) the dogs had it nearly every year and it had greatly decimated them; but the fox epidemics still had a longer periodicity. Symptoms of dogs: froth at mouth, get light-headed, and tendency to run in straight lines. Become very weak, not violent, and die in about three days. Nearly always fatal. Usually in late summer or fall. Mr. Douglas also described what he believed to be a different disease in the dogs. Symptoms: eyes sunk in head, cheeks swollen below eyes, and great running at the nose. This also occurs in the fall. (From W. J. Peters, May 30, 1931.)

Epidemics observed at Baker Lake in 1918-19 and 1919-20, also observed during August 1922 at Chesterfield Post. Symptoms: foaming at the mouth, fighting, biting, madness, running about. Get weak, and usually are shot. (From H. T. Ford, Post Manager, Nonala Post, May 28, 1930.)

Eskimo Point Post

Epidemic in November and October, in 1927 or 1928. Symptoms: foaming at mouth and nose, general weakness and loss of appetite, apparent paralysis of the body. (From T. C. Carmichael, May 10, 1930.)

Evidently the same epidemic referred to by Dr. Hadwen (10) who stated, "there is one northern disease which occurs periodically and which has somewhat the appearance of rabies, but from what the writer has learned about the infection, it cannot be true rabies. Whatever be the exact nature of the disease, it attacks all canines either wild or domestic." Just as the writer had finished his paper he received a letter from the Rev. Marsh of Eskimo Point, Hudson Bay, informing him that a bad outbreak of the above disease

had occurred. The symptoms he gives are as follows: 'The dogs are again suffering from this terrible scourge. It seems to me like rabies. The cheeks swell, the nose stops up, the mouth drips saliva, the animal is gradually paralyzed (from the hind quarters usually) and very soon is dead'. He adds: 'The natives are all losing dogs and you can realize, I know, how serious a proposition this is'."

Port Harrison Post

Great numbers died in spring 1928 to middle of July. (From Post Manager.)

Fort George Post

Great numbers died during winters of 1925 and 1926 before he came to the post. None observed since. Worst in the fall and spring months. Symptoms: foaming at mouth, running around in circles, gradually becoming weaker until they died. Others would commence to shiver and die almost at once, whilst others would turn blind, their eyes becoming entirely white. The latter were usually shot, as the Indians found that they would bite at any obstruction they met with and I understand that a number of children were bitten but with no dangerous results. (From R. Gordon, June 6, 1930.)

Great Whale River Post

The sickness among dogs has occurred as long ago as the oldest Eskimo can remember. Symptoms: some go blind, others go mad and bite, others have fits while some dogs stagger about with their hind quarters useless. The Eskimos shoot any dog that shows signs of the sickness to prevent the disease spreading. (From L. G. Maver, May 10, 1930.)

Moose Factory

One of the Company's Post Managers while in London informed the author that the dog disease had gone all round Hudson Bay, *e.g.*, to Moose Factory, and that it now occurs every year. *Furthermore, that it does not attack non-sledge dogs, e.g., St. Bernard.* That this disease, or something similar, has long been established in the Bay is shown by the experience of Mr. Copley Amory, who informs me that in the early part of 1898, during the winter, there was an epidemic among the sledge dogs, when the teams arrived at Moose Factory from different directions (*e.g.*, some from Great Whale River to the north). The disease was described as "distemper".

In spite of minor disagreements (no doubt due to the different local experiences of epidemics or to generalizations made from rather few observations) the accounts leave no doubt that this sledge dog disease is a definite entity, occurring over a large part of Arctic Canada. The symptoms seem to differ not only in different dogs, but in different epidemics and in different localities. An example of the last phenomenon is the record of occasional blindness at Fort George and Great Whale River, both in southern forest region of the Bay; the emphasis laid on the hanging jaw at Lake Harbor and Frobisher Bay; the curious variations in fierceness or tameness of the dogs, etc. If the disease is a form of encephalitis, as Dr. Hadwen and Dr. Green believe probable, this variability of the symptoms is not surprising, since similar variability is well known in human encephalitis, and in the silver fox encephalitis studied by

Dr. Green. The most constant features seem to be the erratic behavior, foaming at the mouth, partial paralysis and the comparatively swift death, and the great percentage mortality accompanied by great infective power.

There was a very serious dog epidemic along the north shore of the St. Lawrence during the early part of 1930. The information about this epidemic has not yet completely come in and will be reserved for later publication. It seems probable that the disease may have been the same type as that occurring further north, but intensive work is needed before this can be decided. There were also outbreaks in northern Labrador during the winter of 1930-31, for information on which the writer is indebted to Dr. Harrison E. Kennard, who accompanied Dr. Alexander Forbes' expedition to Labrador in 1931. Most of his information was obtained from the Hudson's Bay Company Post Manager, Mr. Haynes, at Hebron. During the winter, a great many dogs were killed with the disease at Cartwright, Northwest River, Rigolet, Hopedale, and Davis Inlet posts, but Okkak and Hebron escaped. Mr. R. Bain in answer to questionnaire, summer 1929, stated that there had been no dog epidemics at Okkak, or Nutak as it is now called, for the past number of years. The symptoms were apparently similar to those described in the questionnaire. It would therefore seem probable that the north shore and the Labrador epidemics were part of the same pandemic. The old records of the Moravian missions in this region (cited by Gosling) reveal the fact that such pandemics have long been a source of loss and suffering along the coasts. "In 1859 the dogs were again attacked by the distemper which periodically visits the Labrador. The cause of this mysterious disease has not been ascertained. It seems to be rather infectious than contagious, for it breaks out simultaneously all over the coast, at places very widely separated and with no communication. The dogs in Ungava Bay were afflicted at the same time as those in Hopedale. It not only attacked the dogs but the wolves, foxes, and even the caribou died in vast numbers from the disease. We seem to know very little about the various pestilences to which wild animal life is subject" (5, pp. 301-302). Again in 1868 the "loss of sense" disease attacked the dogs (5, p. 304). Further back, in the winter of 1836-37 the distress of the hard winter "was very greatly intensified by a distemper among the dogs which causes the death of about 90% of these useful animals" (5, p. 295).

We have then evidence that these pandemics have raged periodically in Labrador for at least a hundred years. It seems that similar epidemics occur in Alaska, since Dr. Hadwen states in a letter to the author: "In Alaska all the men I spoke to believed that the dog and fox disease were one and the same, and the periodicity was as you say, four years." The dog disease is known in the Athabasca-Mackenzie region also: Mr. V. W. West, then district manager of James Bay district, wrote on March 25, 1930 that "in connection with these periodical epidemics the writer has seen several such, some of them occurring in the Upper Mackenzie district and Athabasca, where the dogs are far removed from any danger of being infected by eating the carcasses of

white foxes." The fox disease has also a wide distribution which will be referred to later.

Dr. R. G. Green has suggested to the writer the possibility that the main disease (encephalitis) is complicated either by secondary infections or by the existence of parallel but different diseases such as true distemper, or the paratyphoid also found in silver foxes (8). These questions can be solved only by intensive pathological work along experimental lines.

This paper is concerned chiefly with the mode of origin of the disease or diseases. It is obvious that epidemics might arise in several ways. They may arise spontaneously in dog teams containing the disease organism in a suppressed condition, either through lowered resistance due to food shortage or some other cause. Mr. V. W. West added in his letter that "the writer has further noted that, when such epidemics occurred, they usually commenced among the Indian dogs, which are generally in a state bordering on starvation, while the post and police dogs would be the last to become infected. Furthermore, the percentage of deaths among the Indian dogs would be very much higher than the better cared for dogs of the white people. This may only point out that the undernourished dogs are more liable to get the disease in a virulent form than would be the case with the strong, well-fed dogs. On the other hand, malnutrition may be the direct cause of the disease." It will be remembered in this connection that neither half-breed nor pure non-sledge dogs (*e.g.*, St. Bernard) were attacked in the epidemics at Frobisher Bay and Wolstenholme. In the last two epidemics all dogs were obviously exposed more or less to infection, and were evidently immune for some reason. It would be important from a practical point of view to find out whether this immunity was general (*e.g.*, due to better food and care) or specific (*i.e.*, against this particular disease). It may be noted that the fact of disease in the southern posts starting first in the Indian dogs, might be explained also by the greater contact that these dogs would be likely to have with diseased foxes or other wild animals, although on the other hand the reason may actually be that suggested by Mr. West.

Even if the disease is not derived, in the first place, from the sledge dog population itself, it seems certain that it can be spread to a large extent by contact with outside teams of dogs that have had or are having the epidemic. Although there is, I believe, general agreement that this is so, it would be in practice very important to know to what degree convalescent dogs or immune dogs can act as carriers of the disease, and how long such carriers would require to be quarantined, and what kind of quarantine is necessary or possible. We have next to consider the suggestion that the disease is derived from some other animal—in this case wild animal. This idea is discussed in the sections that follow.

Epidemics in the Arctic Fox

In this section is given a summary of the answers to questions 4, 5 and 6, in the questionnaire sent out to the posts. Names of informants as in last section. Two species of fox are mentioned. First, the arctic fox (*Alopex*

lagopus) known to the fur trade as the "white fox", normally an inhabitant of the arctic regions north of the tree limit, but migrating far to the south in years of food scarcity. It has two color phases, the winter white one, and the much rarer "blue" phase. The other species is the red fox (*Vulpes fulvus* and other subspecies or possibly species) known to the trade more accurately as "colored fox", owing to the existence of several color phases including red, cross, "silver", and black. This species normally inhabits the forest zone and regions to the south.

George's River Post

No epidemic in white foxes seen by him, but one of the natives informed him that a few years ago he saw a white fox suffering from the disease. Symptoms: frothing at the mouth and apparent madness.

Fort Chimo Post

Four dead white foxes in the fall of 1922. Symptoms: similar to those of the dog disease described on p. 676. This information mostly obtained from natives.

Mr. L. Romanet, formerly manager of the Athabasca-Mackenzie district of the Hudson's Bay Company, informed the author in 1928, that he had been at Fort Chimo in the years from about 1908 to 1916, working for Revillons Frères Trading Company. According to his observations the white fox suffers from disease in the spring and summer following the disappearance of the lemmings after their periodic peak in numbers. Symptoms: dizziness and sudden attacks of rabies-like madness. The disease also attacked dogs.

Wolstenholme Post

Had heard of the fox disease from native sources, but had not been able to secure reliable data.

Lake Harbor Post

Had seen a fox epidemic here some years previous in 1926, had found dead foxes under the rocks and seen them running *e.g.*, one ran madly into his house and was killed with a stick. The jaw was hanging in the same way as that of a dog with the disease. (Lake Harbor had its periodic fox peak in 1921, and so this epidemic was probably associated with it. C.E.)

Clyde Post

Older natives remembered disease among both dogs and foxes.

Pond's Inlet Post

Had never seen this disease among foxes, "but according to the natives a severe epidemic did occur about fifteen years ago, when it killed practically all the dogs, foxes, and wolves in the country". All these species died from the same disease. This sickness seemed to be very contagious and spread very rapidly. In recent years no disease has occurred among the foxes at this post.

Repulse Bay Post

Only place he had seen live foxes was in traps, and none of these had shown any sign of disease. Is convinced it must be very rare among them, as he had never heard the natives say anything about the disease, either here or at

Wager Inlet, Baker Lake, or Chesterfield. (This disagrees with some of the accounts given below. C.E.)

Chesterfield Post

Had never seen fox disease personally, but natives stated that several of them had come across foxes, both in winter and summer, acting in a crazy way, and they described it as the same antics that their dogs go through while affected with the above-described disease.

Baker Lake Post

One instance of a diseased fox had been observed, in first week of May, 1930. "Our interpreter was carrying ice from the Lake one day when a white fox ran up and repeatedly snapped at his legs. It finally made off and shortly afterwards ran up to a team of dogs, when it was immediately killed. Apart from the fact that it was foaming at the mouth, there seemed to me to be no similarity in the symptoms shown by the fox to those of the dogs. Its eyes were bright and sparkling, the body and fur in prime condition, and from the fact of its attacking both the man and the dogs, the symptoms showed every sign of being those of rabies" (W. J. Peters).

Mr. Douglas' observations on the periodic fox epidemics have already been mentioned (p. 678). The foxes usually have it every fourth year, and in the spring (end of April or early May). They become light-headed and are easily caught or knocked on the head, but are certainly not in a starving condition.

There was an epidemic in white foxes in the winter of 1917-1918, many being found dead. Symptoms apparently similar to those of the dogs (H. T. Ford).

Fort George Post

Had not seen any disease among white foxes, but the Indians stated that they had seen colored foxes foaming at the mouth and acting just like a dog attacked by this disease. Quite a number of colored foxes were found dead by the Indians and theyappeared to think that both dogs and foxes are attacked by the same disease.

Great-Whale River

Epidemics have been seen in white foxes both in winter and summer. They go mad and bite at anything within reach. Dogs are often bitten by the foxes.

The author believes that most of the information from natives must be in the main reliable, since sledge dog or white fox epidemics have the same importance for the Eskimo in the North, as a big railway accident or a coal strike have for the man in the street in Canada or England, and will be remembered just as easily.

There are the widespread native accounts of epidemics among white foxes (from George's River, Fort Chimo, Wolstenholme, Clyde, Pond's Inlet and Chesterfield Inlet) and among colored foxes at Fort George. Circumstantial stories of the disease in particular cases are given by natives from George's River and Baker Lake. In addition there are the personal experiences of some of the post managers, as at Lake Harbor, Fort Chimo, Baker Lake, and Great Whale River, and the testimony of dog owners in Alaska (given by Dr. Hadwen (see p. 680). There seems no reasonable doubt, therefore, that

white foxes in the north and (to some extent, at present unknown) colored foxes in the forest belt to the south, are liable to outbreaks of epidemic disease (usually resembling encephalitis) similar to that which attacks sledge dogs.

The area covered by this evidence includes a large part of Baffin Island, the shores of Hudson Strait, Ungava Bay, and the northwest part of Hudson Bay, also posts to the south of this, on the east side of James Bay. Since white fox disease occurs also in Alaska, it seems likely that it will be found to occur right across the Western Arctic of Canada, in the intervening region. It is of great interest to note that a similar disease may occur in Kamchatka. The author is indebted to Dr. Sten Bergman of Stockholm for amplifying the information on this subject given in his book on Kamchatka. In the book he states: "Tracks of foxes and sables were very rare that winter. During the previous year there had been an epidemic among these animals, and it had reduced their numbers to an incredible extent. The foxes which used to be extremely numerous here, had now almost entirely disappeared. In every village one heard complaints of the poor hunting." (1, p. 128).

This information refers to the colored foxes, although white foxes do also occur in the northern part of the peninsula. Dr. Bergman wrote to the author: "The exact date of the winter in which sables and foxes were scarce was 1920-1921. During that winter they were extremely scarce; I never crossed any traces of sables, and only a few of foxes, and all hunters complained of the bad hunt. The previous winter 1919-20 they were so abundant that they could be caught in the villages without any difficulty, and the inhabitants told me very often that the foxes had some disease and that they could be killed very easily with a stick as they often had lost the fear of man. I remember several kamchadals telling me that they had killed foxes when dog-driving only with the stick ("ostol") used as a breaker for the sledge. They said that the foxes often during the winter 1919-20 would run straight against a dog-team, and that they died in the woods. Sables could be caught in the villages and several inhabitants told me they had caught sables among the firewood near their houses. All said they had some disease. I arrived in Kamchatka in the summer of 1920 so I could not see the abundance myself. Of the three summers I spent in Kamchatka, mice were extremely abundant in the autumn of 1921, but not in 1920 and 1922. Hares I found very numerous both the winters I spent there (1920-21 and 1921-22). But I received a letter some weeks ago from a Swedish friend in Kamchatka, and he told me that during the winter of 1926-27 hares and mice had quite disappeared and that the sable was extremely rare." The notes on fluctuations in rodents are in themselves of great interest in showing that in Eastern Asia there are fluctuations in the animal population similar to those found in Alaska and Canada among hares, mice, and other animals. They are of particular importance here in suggesting that the deaths among foxes were not due directly to starvation, since there were enough hares, (and probably mice too ?) to provide them with food.

Similar evidence about the epidemic which attacks the arctic fox in Canada is provided by the statements of Mr. V. W. West and Mr. G. R. Ray, officials of the company who have had long experience in Canada and who

informed Mr. Binney that they could recall instances in exceptionally good white fox years when they have found many dead foxes. Death they stated was not due to starvation, the foxes being plump, but to "rabies". Snapping and snarling, they run round in circles with froth exuding from their mouths and finally fall down palpitating and die. There was an instance recorded where a missionary had the heel of his boot bitten off by a white fox in this condition. It was also stated that coyotes, wolves, and Eskimo dogs likewise contract this disease.

It is important to note that wolves are said to suffer from the same epidemic as white foxes. Statements to this effect were made by several observers already noted and by Gosling, in his account of the Labrador Moravian Missions (see p. 680). It would be very important to find out how far such epidemics act as a check upon the numbers of wolves, in view of the latter's great influence upon the caribou herds and upon other wild animals, not to speak of their direct value as fur-bearers. Gosling mentions a caribou epidemic in 1859. It would be of immense importance to know if this were really caused by the same organism which attacked the dogs, wolves and foxes in that year.

It may be concluded then, that white and colored foxes are both liable to periodic epidemics, sometimes on a very large scale, over the whole of the arctic and subarctic regions between the longitudes of 60° and 160°—in fact a third of the way round the north of the world. Before considering the relation of dog epidemics to the sledge dog epidemics it is necessary to go into the question of cycles in numbers of the arctic fox, which have an important influence on the periodicity of the disease and also on the origin of it.

Cycles in Numbers of the Arctic Fox

It is well known that the arctic fox is subject to very violent fluctuations in numbers which are sufficiently regular to merit the name of cycles. Years of great abundance are followed by rapid decrease to great scarcity, after which the numbers increase once more to a peak. This cycle is reflected in the Hudson's Bay Company's London sales: the curve for the annual number of white fox skins sold between 1850 and 1914 is to be found in Hewitt's book (11). In 1924 the author published an investigation of this cycle, and pointed out that the periodicity was one of about four years (a fact already noted by Hewitt), and that since this periodicity was the same as that of the Norwegian lemming (*Lemmus lemmus*) and since Canadian arctic foxes are supposed to depend for food upon lemmings, amongst other things, it was probable that the white fox cycle of the Canadian north could be referred back to a similar cycle in numbers of lemmings. This suggestion received support from the general agreement of the actual years of lemming abundance in Norway, and those in Canada, as deduced from the fox curve. In 1925, in another paper, it was shown that more recent white fox statistics (derived from Canadian Government royalty returns) carried the agreement up to 1922. Since that time, investigations carried through the Hudson's Bay Company and elsewhere have thrown a good deal more light on the problem. This work will be reviewed below in so far as it bears upon the periodicity of white fox epidemics: it opens

up a large number of other problems which cannot be dealt with here, and will be reserved for publication elsewhere.

Periodicity

The curve already published (2, 3, 11) represents the catch of white foxes for the whole of the Hudson's Bay Company's area of operations; in it is included the catch in posts to the south of the barren grounds and arctic regions that form the white fox's usual habitat. In the Hudson Strait area all fox skins in the earlier period came from Ungava Bay (Fort Chimo), and it was not until 1909 onwards that the white fox trapping industry was established on an extensive scale in the north, with the creation of a large number of new posts. Fort Chimo, which was established in 1828, is therefore the only post which can supply a long series of statistics from the arctic regions where the fox breeds permanently. Wolstenholme Post was established in 1909, Lake Harbor and Chesterfield Inlet Posts in 1911, followed at intervals by some 27 other posts over the whole of the eastern Arctic. Fur statistics from Fort Chimo are available from 1868 onwards, but those for one or two years are missing. If we study the continuous record since 1881, we find that the years of periodic maximum numbers were 1882, 1887, 1890, 1893, 1897, 1901, 1905, 1909, 1913, 1917, 1921, and 1926. (These dates are not the actual years in which the returns were made, but refer to the previous year: the *biological production* of white foxes takes place in the spring and summer months, the foxes are trapped in the following winter, and returns are therefore a year later than the production. In comparing lemming abundance with fox abundance, it is obviously simpler to deal with the year of actual fox production in nature rather than the year in which their skins attain commercial interest.) It will be seen that the interval between maxima is four years in seven instances, five years and three years in two instances each; the average periodicity is therefore exactly four years. There was an undoubted maximum year in 1872; certain records are missing between 1872 and 1882, but if we assume that a total of 14 cycles occurred between 1872 and 1926, the average periodicity now works out at about 3.9. It is clear that there is some very regular influence at work producing a tendency to an almost exactly four year cycle (varying occasionally to three years or five years), and which has been going on at any rate since 1872. The scarcity of white foxes in the North in 1928 was part of this periodic cycle, which has therefore continued for nearly sixty years.

This periodic cycle in white foxes is not always absolutely synchronous over the eastern Arctic. In some years (as in 1926) practically the whole of Baffin Island, Hudson Strait, and the arctic parts of the Bay have the same maximum. In 1921 a region in the southeast half of this area (Frobisher Bay, Port Burwell, Lake Harbor, Stupart's Bay, Wolstenholme, and Port Harrison) had its peak year, while the other half of the whole area (including Pangnirtung, Amadjuak, Cape Dorset, Chesterfield Inlet, Baker Lake, and Eskimo Point) had its maximum year in 1922. Again, some posts reached a peak in 1917 (as Fort Chimo did), while others reached it in 1918. The cycle is therefore not sufficiently hard and fast to be used for anything but general forecasting

as it stands, since the peak years may be single or in couples: on the other hand, the regularity over long periods, and the regional character of the years of abundance, and the resemblance to similar cycles in Norway, open up avenues of enquiry which should lead to a real understanding of the factors controlling the cycle.

Causes of the Cycle

It is well known that the arctic fox in summer is mainly dependent for its food upon lemmings and ptarmigan. While it certainly supplements these with any other animals it can obtain (particularly on the coast, where eggs and young sea-birds, offal, etc. may be found), it seems fairly certain that over the vastly greater part of its breeding ground, lemmings and birds are the main source of food, and therefore the main controlling factor in the summer breeding of the foxes.

There are only scattered data dealing with the question of lemming cycles in Canada, and the phenomenon can be best illustrated by what is known of lemmings and white foxes in Norway. The author has shown (2, 3) that if the periodic migrations of the lemmings from the Norwegian mountains are taken as an index of periodic overpopulation due to increased numbers, it appears that there have been migrations in south or central Norway almost every four years since 1862 at any rate. The actual average periodicity between the migration years is 3.9 years. In estimating this periodicity it should be noted that two years of abundance were *assumed* to exist, although no records of migrations exist. The justification for this method is proved by examination of the statistics for foxes (based on annual Norwegian Government bounty statistics) recently published by Johnsen (12). The fox curve includes both the skins of arctic and of red foxes: it shows a remarkable resemblance to the Canadian record. The maximum years of foxes (years of production, not of bounty payment) are very regular, and agree with the lemming records. There are peaks shown in the years for the two missing records of lemming migrations (implying that comparatively high numbers were reached but did not lead to migration). The fox period, *e g.*, for the Nordland District of Norway since 1880, averages exactly four years. In Norway, cycles also take place among wild mice and voles, generally in agreement with the lemming cycle, and they are an important additional, in some cases main, factor affecting fox numbers. The same is no doubt true of some of the southern parts of arctic Canada, where voles are an important element in the barren ground fauna. As regards ptarmigan, it is interesting to note that during part of the nineteenth century there was a regular cycle in ptarmigan numbers in Norway, agreeing with the lemming cycle (13). There is also evidence of a short ptarmigan cycle in arctic Canada, but not enough to determine whether it exactly synchronizes with that of the lemmings. It is clear that a short cycle in lemming and ptarmigan numbers in Canada is the most likely explanation of the main cycle of white foxes. This hypothesis has been confirmed for certain localities. The writer does not propose to follow up this problem further in the present paper, as a very large amount of evidence connected with rodent cycles would have to be detailed and discussed.

Although the connection between foxes and lemmings may explain part of the cycle, there still remains the problem of the regional nature of the cycle, and the agreement of widely separated areas in the same years. The same question is raised by the Norwegian vole-lemming cycle, which synchronizes not only all over Norway but also with the four-year cycle in British voles worked out by Middleton (14, 15). It is difficult to escape the conclusion that some climatic factor is involved, *e.g.*, variation in the annual amount of snow cover in the winter, of the wetness or dryness of vegetation, of temperature, etc. Research on this phase of the problem is being actively carried on at present. It is clear, however, that although this research may seem to carry us very far from the original problem of sledge dog epidemics, in reality all these different subjects are intimately connected. If the sledge dog epidemics are connected with arctic fox disease, and the latter with the arctic fox cycle, forecasting of either will be dependent to a great extent on a knowledge of the causes of the fox cycle, which appear to lie with similar basic cycles among rodents and game-birds, both in turn dependent, in all probability, upon diseases and upon some climatic influence acting rather regularly over very wide areas of the Canadian and European Arctic. Climate may also be found to produce a direct cyclical influence on the arctic fox. To attain a complete understanding of this chain of events, we should have to determine the nature of the climatic factor and seek the causes of its variation in terrestrial or astronomical processes. The discovery of all these different processes will at the same time throw a great deal of light on fur trade problems and upon similar cycles in other countries.

Disease in Relation to the Fox Cycle

It is believed by a good many men living in the North that the disease attacks white foxes at the time when lemmings have disappeared, food is short, and the fox unable to resist disease. Exact information on this subject is unfortunately very limited. The replies to questionnaires give very little data about the exact years of fox epidemics, although the season is mentioned. Apparently diseased foxes have been found at various times of the year. One exact record (Fort Chimo, fall of 1922) fits in with the cycle, since the foxes were at their peak there in 1921, and would be decreasing during the following spring and summer. The other (Baker Lake, May, 1930) must await more information about recent movements of the fox cycle.

It is therefore not possible to give any conclusive statement about the periodicity of white fox disease; at the same time it seems highly probable in light of what has been said about the fox cycle, and of the general testimony of men who have had experience on the spot, that the disease does usually happen at the end of the winter or in the summer following the year of maximum numbers. There is an important possibility to note: in Norway the sudden decrease of the lemmings is caused by virulent epidemics (and also to an important extent by deaths on migration). These lemming epidemics have been studied bacteriologically in certain cases (literature summarized in (4)).

There is a well-known disease among human beings in the Norwegian valleys

called "lemming-fever", because it is supposed to be derived from water polluted by dead lemmings. This disease is also said to attack domestic animals. The whole problem has never, however, been adequately investigated. It is mentioned here to show that the periodic epidemics of the lemming can probably infect other animals at the same time. This opens up the possibility that the white fox disease is not only caused by the condition of the fox, but may even be derived directly from lemming epidemics. It is perhaps far-fetched to suggest that the migratory tendency of lemmings (attested in Canada by numerous observations which cannot here be summarized) is an expression of some nervous disease. In particular the tendency to move about in rather straight lines, and refusal to alter their direction, and the fact that they behave sometimes in quite a mad and savage manner (they will sit up and fight a man) also might be explained in the same way. This suggestion may however emphasize what may only be a casual coincidence in behavior due to quite different causes. At present we have no definite evidence that lemmings actually do have epidemics of infectious disease in the Canadian Arctic. The supposition is that they do, since the reduction in numbers during a single winter may be so very sudden and great.

It has been suggested to the author by several people that lemmings are never sufficiently numerous to control the arctic fox population. In this connection it is important to note two things: in the first place, that Norwegian lemmings are mainly nocturnal, and unless they are migrating, do not show themselves very much in the day. The great difference between a migrating lemming in Norway, and the individuals of the same species when "at home", was noted by the writer in Norway last summer. It is possible to search for lemmings in the day and find none, even when they are present in fair numbers. The second point is that the numbers of arctic foxes are probably exaggerated, since they tend to be focussed at certain points in winter, especially when they are migrating.

Migration

The arctic fox undertakes tremendous migrations, chiefly in the winter and spring. After a year of abundance in the North, it is common for a "run" of arctic foxes to be met with in the posts further south, in the margin of the forest zone, and actually also right down into the heart of this region. The extent and duration of these migrations south vary considerably. They are important for the present discussion, in so far as diseased white foxes are liable to infect dogs and wild animals south of their usual range.

Epidemics Among Red Foxes

While the white or arctic fox is normally an inhabitant of the northern regions, the forest belt is the normal home of the colored fox or red fox (including a group of species each containing the varietal phases of red, cross, silver, black, etc.). On examining the replies to several hundred questionnaires from the forest belts of Canada, extremely few records have been found of epidemics among colored foxes, although the period of investigation happened to cover the decrease of foxes following the periodic decrease of rabbits on their ten-

year cycle. This may be due partly to lack of observation, but cannot be due entirely to that since a good many epidemics among rabbits, mice, muskrats, etc. were mentioned in the answers. We may probably conclude that natural epidemics in colored foxes are far less common than they are in the arctic fox. One of the records of colored fox epidemics is accounted for by the theory that they had caught the disease of the white fox. Thus a reply from Great Whale River Post stated that "the Eskimos at the Belcher Islands report a number of white and colored foxes were found dead last summer (1927)." Two others cannot apparently be accounted for in this way. A reply from Island Lake Post, N.E. Manitoba, (G. C. M. Collin), stated that "I have talked with an Indian who states he has come across carcasses of dead foxes. He states they were in a very emaciated condition; it must be sickness as rabbits were plentiful in this locality. This was during the winter of 1929-30." Another report from Fort St. James, British Columbia, stated that there was an epidemic among colored foxes and prairie wolves between October 1927 and January 1928. Finally, the experience of silver fox farmers in Minnesota proves that encephalitis and other diseases are endemic among the silver foxes brought into captivity, unless of course they are derived from dogs.

Relation of Dog and Fox Epidemics

In those instances where both fox and dog epidemics have been observed in the same locality there is usually agreement on the part of observers that the two diseases are identical. Mr. Blackhall (Fort Chimo) stated, "It is the firm belief of the natives that it is the same sickness which attacks both dogs and white foxes. Further they believe that a close connection exists in the scarcity or abundance of lemmings in the district. When lemmings have reached their peak, foxes soon begin to decrease, and also dogs become more or less sick, showing the symptoms as described." Mr. Romanet had arrived at the same conclusion. Mr. Ford, speaking of the winter epidemic at Baker Lake in 1918-19, stated that "it is probable that the dogs were infected through eating dead foxes". Mr. Maver (Great Whale River) reported that "Eskimos state that when mad foxes bite the dogs, the latter at once are attacked with the disease and the native is positive that the sickness among the dogs originated in the white fox".

A simple comparison of the dates of the sledge dogs epidemics already recorded and those of the years of fox abundance (assumed here to be indicators of the probability of fox epidemics) shows no marked connection between the two. But, owing to the fact that teams of dogs may spread the disease from one post to another, it is not expected that any such simple relation would be found. We should expect that periodically there might be outbreaks of disease derived from foxes, which would lead to a succession of dog epidemics at intervals, probably trailing on until the next fox year. There is also the strong likelihood that recovered foxes may continue to act as carriers of the disease, thus carrying on the epidemics, as with silver foxes on farms (6). It is also clear that theoretically the fox disease might be derived from the dogs. This seems very unlikely. Or the two may be independent. This also seems unlikely;

unless the dogs depend to an important extent on wild rodent food in summer, and become infected by it. Mr. Douglas makes the important observation that the disease was formerly periodic (four years) at Baker Lake, but now occurs every year in the dogs. This indicates that in some places it has become endemic among the dogs themselves. In other areas however (as at Fort Chimo) the disease certainly does not occur every year. It is interesting to note the following statement from the manager of that post: "We are informed by our interpreter, Thomas Gordon, who has been a servant of the Company for over forty years, that it has always been an unusual thing to rear successfully young dogs on the Post, and can give no satisfactory explanation how this should be, beyond saying that he himself also experienced the same." It is suggested that *the wrong food* is the reason. A population of dogs not recruited through puppies would have different reactions to disease than one into which susceptible young dogs were constantly introduced. There appears to be a strong and growing body of evidence pointing to the disease being endemic and breaking out annually at posts on the northwest corner of Hudson Bay, but being periodic at other parts.

Summary

1. Disastrous epidemics occur periodically among the sledge dogs of the Canadian Arctic. The results of an enquiry conducted by the Hudson's Bay Company among their Posts in Baffin Island, Hudson Strait, and Hudson Bay, are recorded and discussed.

2. The commonest disease attacking the dogs resembles the "fox encephalitis" found on silver fox farms in the United States. There may be other diseases associated with this one.

3. Half-breed and non-sledge dogs are partially or completely immune to the disease.

4. A similar and probably identical disease occurs among arctic (white) foxes in the North, and appears to be associated with the periodic cycle in numbers of the foxes. Occasional records have also been made of disease in colored (red, cross, silver) foxes in the forest region of Canada, and these are possibly derived in some cases from the arctic fox. Wolves and caribou are also said to be attacked by the disease.

5. An intensive pathological study of the disease, both in dogs and in wild foxes, is required, and would probably lead to the discovery of some means of immunizing sledge dogs against the disease.

6. General forecasting of the fox epidemics is now possible. The development of this aspect of the subject depends upon a study of the rodent cycles which probably control the fox numbers, of the diseases of lemmings, and of the climatic factors which bring about simultaneous abundance and scarcity of lemmings and foxes over large areas of the Arctic.

7. If the dog disease is ultimately derived from the arctic fox, the large-scale migrations of the latter into regions south of their normal range assume a great importance, since migration appears to take place at the same time as outbreaks of disease in the foxes.

8. There is some evidence that the disease is establishing itself permanently in the dog population, *e.g.*, on the northwest shore of the Hudson Bay. In this connection a careful study of the age distribution of the disease in dogs, and of the occurrence of healthy carriers among dogs and foxes, is desirable.

9. The whole problem of sledge-dog epidemics has an extremely important bearing upon the economic life and welfare of the natives, and through them upon the prosperity of the white population in the North and elsewhere.

References

1. Bergman, S. Through Kamchatka by dog-sled and skis. Lippincott. 1927.
2. Elton, C. Periodic fluctuations in the numbers of animals: their causes and effects. Brit. J. Exptl. Biol. 2: 119-163. 1924.
3. Elton, C. Plague and the regulation of numbers in wild mammals. J. Hyg. 24: 138-163. 1925.
4. Elton, C. The study of epidemic disease among wild animals. J. Hyg. (In press). 1931.
5. Gosling, W. G. Labrador, its discovery, exploration and development. Toronto.
6. Green, R. G. Epizootic encephalitis of foxes. II. General considerations of fur-range epizootics. Am. J. Hyg. 13: 201-223. 1931.
7. Green, R. G. and Dewey, E. T. Fox encephalitis and canine distemper. Proc. Soc. Exptl. Biol. Med. 27: 129-130. 1929.
8. Green, R. G. and Shillinger, J. E. Results of research on diseases of fur-bearing animals in captivity. J. Am. Vet. Med. Asscn. 74: 277-282. 1929.
9. Green, R. G., Ziegler, N. R., Green, B. B. and Dewey, E. T. Epizootic fox encephalitis. I. General description. Am. J. Hyg. 12: 109-129. 1930.
10. Hadwen, S. The sled dogs of the north country. Saskatoon Daily Star, March 10, 1928.
11. Hewitt, C. G. The conservation of the wild life of Canada. Scribners. 1921.
12. Johnsen, S. Rovdyr- og rovfuglstatistikken i Norge. Bergens Museums Aarbok. 1-118. 1929.
13. Kloster, R. Veksling i rypebestanden. Norske Jaeger og Fisker Forenings Tidsskrift. 50: 317-332. 1921.
14. Middleton, A. D. Cycles in the numbers of British voles (Microtus) J. Ecol. 18: 156-165. 1930.
15. Middleton, A. D. A further contribution to the study of cycles in British voles (Microtus) J. Ecol. 19: 190-199. 1931.

THE CONSTANCY OF REPEATED AGGLUTINATION TESTS IN THE DIAGNOSIS OF PULLORUM DISEASE[1]

By Jacob Biely[2]

Abstract

A high degree of consistency was secured in retests of five groups of birds from various sources and tested from 2 to 22 times. The results of repeated agglutination tests were, except in a few cases, confirmed by the macroscopic appearance of the ovary and by bacteriological examination.

Data show that positive reactors consistently react positive to the test, and that they seldom recover from pullorum infection. This is especially true of birds that have completed the first laying year. With these very little variation can be expected in retests at short intervals. In the case of pullets that are just starting to lay, a small percentage of reactors may throw off the infection and subsequently react negatively.

Non-reactors from an infected flock when left in contact with reactors in presence or absence of males, may in later tests react positively. This is generally due to infection through contact with infected birds, contaminated droppings, feed, water or litter. These non-reactors, if kept isolated from reactors, as a rule remain negative. When such birds become reactors at subsequent tests, the possibility of recent infection taking place just before or after the first test is not excluded. Hence, non-reactors from infected flocks should be retested at short intervals.

Suspicious reactors as a rule do not show marked variations in titre from month to month. It is impossible to predict whether a suspicious reactor will in course of time become a distinctly positive or negative reactor. With these birds therefore diagnosis must be done with care and the general condition of the flock taken into consideration. In an eradication program the bird has to be sacrificed unless it is very valuable, in which case several retests would need to be conducted before a final diagnosis is made.

Male birds do not as a rule react in as high dilutions as females, consequently fluctuations in reaction from test to test are not uncommon. Therefore, particular care must be exercised in diagnosis in the case of male birds.

Introduction

The agglutination test for pullorum disease introduced by Jones (18) in 1913 has been widely applied to commercial breeding flocks as a measure of control. Until recently it was generally believed that annual testing of flocks, and the elimination of reactors discovered at each test, would eventually result in the eradication of the disease. However, investigations during the past few years point towards the necessity of retesting flocks at short intervals (6-8 weeks), in order to ensure more rapid and certain eradication.

In a series of papers published since 1927, Beach, of Wisconsin, and his associates (1, 2, 33, 34, 35) drew attention to the variability of repeated agglutination tests and the consequent difficulties encountered in eradicating pullorum carriers from an infected flock. A similar report has been simultaneously published by Beach of California (3). The results obtained by Kernkamp (20) agree with those of the two previously mentioned investigators. Quite recently Lerche (23, 24) has reported that there was considerable disagreement between repeated agglutination tests and autopsies of non-reactors and reactors.

[1] *Manuscript received June 25, 1931.*
Contribution from the Departments of Bacteriology and of Poultry Husbandry, University of British Columbia, Vancouver, Canada. with financial assistance from the National Research Council of Canada. This work was carried on under the direction of Dr. H. W. Hill, Head of the Department of Bacteriology, and Prof. E. A. Lloyd, Head of the Department of Poultry Husbandry.
[2] *Research Assistant, Department of Poultry Husbandry, University of British Columbia. Vancouver, Canada.*

Failure of reactors to react to one or more tests and fluctuations in the titres of reactors have been reported by Ericksen (13), Gwatkin (15), Doyle (9), Rice (28), Fitch and Lubbehusen (14), Newsom *et al* (25), Dearstyne *et al* (8), Bushnell and Brandly (7), and Tittsler *et al* (31).

Remarkably uniform results in repeated agglutination tests have been reported by Kaupp and Dearstyne (19), Edwards and Hull (12), Runnells (29), Rettger, McAlpine and Warner (27), Sawyer and Hamilton (30), and Biely, Sawyer, Hamilton, Johnson and Dickinson (5). The results of the repeated agglutination tests reported by the last group were in close agreement with the macroscopic appearance and bacteriological examination of the ovaries of reactors and non-reactors.

In the course of our investigations of pullorum disease in chicks and breeding stock, ample opportunity presented itself to determine the practical value of the agglutination test (4). In order to gain further information on the reliability and constancy of the agglutination test *per se* the present study was undertaken.

Material

The data presented in this paper are based on a study of five distinct groups of birds.

Group 1

This group, consisting of 98 White Wyandotte females (11 to 12 months old), was secured from a flock in which a heavy infection with pullorum disease was known to exist.* The birds were confined in a new building and placed in two separate pens, 12 by 16 ft. Pen 1 included 48 birds and Pen 2, 50 birds. Three cockerels were kept in each pen. The care and management of the birds remained constant throughout the experiment.

The birds were placed in the respective pens on May 1, and none, except those that died, removed until October 1. On October 1, 20 non-reactors were withdrawn from Pens 1 and 2 and divided equally into adjoining pens numbered 3 and 4, each 4 by 16 ft. The birds in Pen 3 were mated to a reacting cockerel and those in Pen 4 to a non-reacting cockerel.

Group 2

This group, which replaced the 20 non-reactors in Pens 1 and 2, was comprised of 12 birds that were known to be reactors. Ten of these were White Wyandotte hens and two Light Sussex hens, all 18 to 20 months old, obtained on November 5 from a flock to which the routine agglutination test had been applied.

Group 3

Thirteen two- to three-year old S.C. White Leghorn hens were secured from an infected flock 10 weeks after the application of the routine agglutination test by another laboratory. These birds were kept in a separate pen 4 by 16 ft. (No. 5) adjoining Pen 4.

**Information obtained through the courtesy of Dr. E. A. Bruce, Animal Pathologist, Health of Animals Branch, Agassiz, B.C.*

Group 4

This group consisted of 396 pullets of six breeds hatched during the season of 1927 from a reacting flock. These birds were hatched, raised and kept under similar conditions. During the first laying year the management and care of all the pullets was exactly the same.

Group 5

Eighteen cockerels of various breeds were obtained from several flocks. Some were mated at intervals with the females of Groups 1, 2 and 3.

The birds in each group were taken at random, shortly before or after the application of the first agglutination test, and thus were not specially selected individuals. Each group, however, represented a distinct flock, the history of which both before and after the application of the agglutination test was known.

Method of Testing

With the exception of the birds in Group 4, which were tested only three times, all were tested at monthly intervals, except as noted below. Blood from the ulnar vein was collected in a sterile serological test tube, which was kept level until the blood congealed and a firm clot was formed. As soon as the bleeding was completed the blood samples were taken to the laboratory, unpacked and placed in suitable racks at once.

As a rule the blood samples were allowed to stand from two to three hours at room temperature and subsequently placed in the ice chest overnight. If the blood samples were taken early in the morning, some of the tests were conducted the same afternoon; if taken late in the afternoon, the tests were conducted early next morning. The tests were thus conducted 5 to 24 hr. after the blood samples were drawn. With few exceptions the sera were always in excellent condition.

Technique of the Tube Agglutination Test

Preparation of antigen

(a) *Media*: 1.5% plain nutrient agar+0.5% peptone; pH 7.0-7.2. (b) *Cultures*: 4 strains of *Salmonella pullorum* originally obtained about 1919 from the Massachusetts Agricultural Experimental Station. (c) *Incubation*: 48 hr. at 37° C. (d) *Physiological salt solution*: Once-distilled water plus 0.85% of c.p. NaCl. (e) *Preservative*: 5 cc. of c.p. phenol to 1000 cc. of physiological salt solution. (f) *Antigen*: The culture growth is washed off with phenolized physiological salt solution and is known as stock antigen.

The stock antigen is diluted 1 part of antigen to about 15 parts of physiological salt solution (no phenol), adjusted to 0.5 turbidity on the McFarland nephelometer, and is then known as standardized antigen. Each lot of fresh antigen is then checked with a previous lot of standardized antigen, and both lots are checked against known positive and negative sera.

Previous to making the dilutions with the serum, 1 cc. of 1% c.p. NaOH solution is added to each 100 cc. of the standardized antigen.

Serum

Only clear sera are used. Hemolyzed samples are marked on the report sheet with the letter "H".

Dilutions

To make a 1:50 and 1:100 dilution—to 0.04 cc. and 0.02 cc. of serum respectively, 2 cc. of the standardized antigen are added.

Incubation

The dilutions are incubated for 24 hr. at 37° C. and held for 24 hr. at room temperature.

Recording of reactions

Symbols used are as follows: +++, ++ = positive reactions; ++ ? = suspicious reactions; − + = negative reactions.

Diagnosis

A reactor is a bird whose serum agglutinates *Salmonella pullorum* antigen in a dilution of at least 1:50, showing either a +++ or ++ reaction (complete or partial agglutination). A questionable ++ reaction in a dilution of 1:50 or a +++ reaction in a dilution of 1:25 is considered as indicating a suspicious or doubtful reactor.

Rapid Method

Antigen

Antigen is prepared in a manner similar to the tube agglutination antigen except that a solution containing 12% NaCl is used and the antigen is standardized to 50 times 0.75 turbidity on the McFarland nephelometer. Each lot of fresh antigen is checked with an old lot of antigen and tested against known positive and negative sera.

Serum

As for tube antigen.

Dilutions

Serum (0.04 and 0.02 cc.) is deposited on a glass plate ruled in 1½-in. squares. Antigen (0.2 cc.) is added to the respective quantities of serum and stirred.

Incubation

Tests are incubated for five minutes at 37° C. First reading of tests is made at the completion of incubation period; second reading, two to three minutes after exposure to room temperature after incubation.

Recording of reactions

Same as for tube method.

Diagnosis

In making a diagnosis consideration is given to degree of reaction and the time required for the reaction to occur.

In conducting the experimental tests five dilutions were employed; *viz.*, 1:10, 1:25, 1:50, 1:100 and 1:200. After October 1 the tube agglutination method was checked by the rapid method. Previous experience with the

rapid method showed that it was equally as reliable as the tube method in detecting carriers.

The method of conducting the agglutination tests remained constant throughout the course of the experiment. The tests were conducted at approximately 30-day intervals, except in June 1927 and July 1928, when the tests were made at 14-day intervals. No test was made in February 1928. Thus from May 1, 1927, to January 25, 1929, inclusive, 22 tests were conducted.

Post-mortem Examination

All surviving birds of Groups 1, 2, 3 and 5 were killed at the end of the experiment and post-mortems conducted. Birds that died in the course of the experiment were usually examined on the same day. Note was made of the general condition of the viscera, whether the birds were in lay, the macroscopical appearance of the ovary, and the presence or absence of *S. pullorum*. In making a bacteriological examination of the ovary at least five or six abnormal ova were cultured on agar slants. In the case of birds not in lay, the dormant ovary was crushed in a sterile crucible and smears made on slant agar. The ovaries of some birds were crushed and sown completely into broth, and plated or transferred on agar slants.

These cultures were stained by Gram's method and if Gram negative were plated. Several colonies were picked from each plate and were subsequently planted in dextrose, maltose, mannitol, lactose, sucrose and xylose broth. Brom Thymol Blue was used as an indicator and 1% of each of the sugars was added. The carbohydrates were sterilized by the intermittent process for three days and checked for sterility. The majority of the cultures were checked against positive serum from natural reactors.

Experimental

The details of the monthly retests and various dilutions are too extensive to be presented in full. The data are therefore summarized in the tables. In order to facilitate further the discussion of the data, each group of birds is considered separately.

Results

Group 1

The birds were tested for 20 consecutive months and the results are shown in Table I. All surviving birds were tested 22 times, while records of 2 to 21 tests are available of those birds that died in the course of the experiment.

Post-mortem examination of 27 of the 34 birds that reacted consistently negative showed perfectly normal ovaries, while two birds contained two to three flabby ova; one had one hemorrhagic ovum; one was affected with dropsy and had several flabby ova; and one had blackish cysts attached to the ovary. Although the ovaries of these five did not appear absolutely normal, none showed the presence of typical lesions of pullorum disease. A careful bacteriological search for *S. pullorum* was negative in all cases. Two were not subjected to post-mortem examination.

TABLE I

SUMMARY OF REPEATED AGGLUTINATION TESTS OF 98 WHITE WYANDOTTE FEMALES CONDUCTED AT 30-DAY INTERVALS

No. of times tested	No. of birds consistently negative	No. of birds consistently positive	No. of birds N/P*	No of birds suspicious or showing fluctuations
22	22	19	10	8
21			1	
20		1		
18		1		
16	1	5		
15	1	2		
13		2		
12	1	5		1
11				1
10	2	2	1	
7	2	2		
6	1			
3	1	1		
2	3	2		
TOTALS	34	42	12	10

**N/P* = *Negative at the beginning of the experiment, but positive at later tests.*

NOTE:—*Fortnightly tests were conducted in June 1927 and July 1928. No test was made in February 1928.*

For post-mortem results see preceding text.

Post-mortem examination of 41 of the birds which reacted consistently positive showed in 40 the presence of typical lesions. In some, 90% of all ova were abnormal, while in others only a few appeared diseased. It is interesting to note that 7 out of the 41 birds were laying internally, and that one had pericarditis. *S. pullorum* was isolated from 39. In the case of the two from which *S. pullorum* was not isolated, one bird laid internally, and the plates were overgrown with contaminants, while the cultures of the other appeared sterile.

Ten of the birds indicated in Column 4, Table I, were in Pen 1, and 2 in Pen 2. The change in the reaction from negative to positive occurred during the following months:— *Pen 1:* June, 1; July, 1; August, 3; September, 3; and January, two birds. *Pen 2:* October, 1; and April, one bird.

Since there were originally 25 and 23 non-reactors in Pens 1 and 2 respectively it is obvious that there was a difference in the rate of spread of infection. It should be noted that one of the two reactors (Pen 2) which showed a change in reaction from negative to positive, did not actually react positively until six months after she had been removed, with 19 other non-reactors, to Pens 3 and 4 as noted above. Since these 20 non-reactors were kept in the same building as the reactors, infection of the bird after removal from direct contact with reactors is not excluded. No spread of infection was observed in Pen 3, in which the non-reactors were mated to a strongly reacting cockerel (from October 1927 to February 1929).

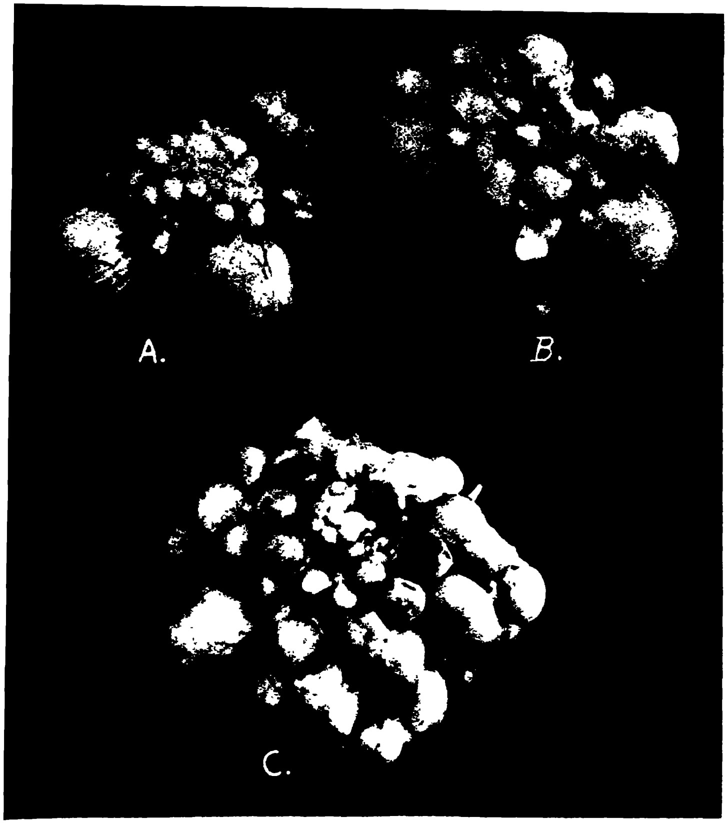


FIG. 1. *Normal and S. pullorum infected ovaries. A.—normal. B, C,—typical S. pullorum diseased ova.*

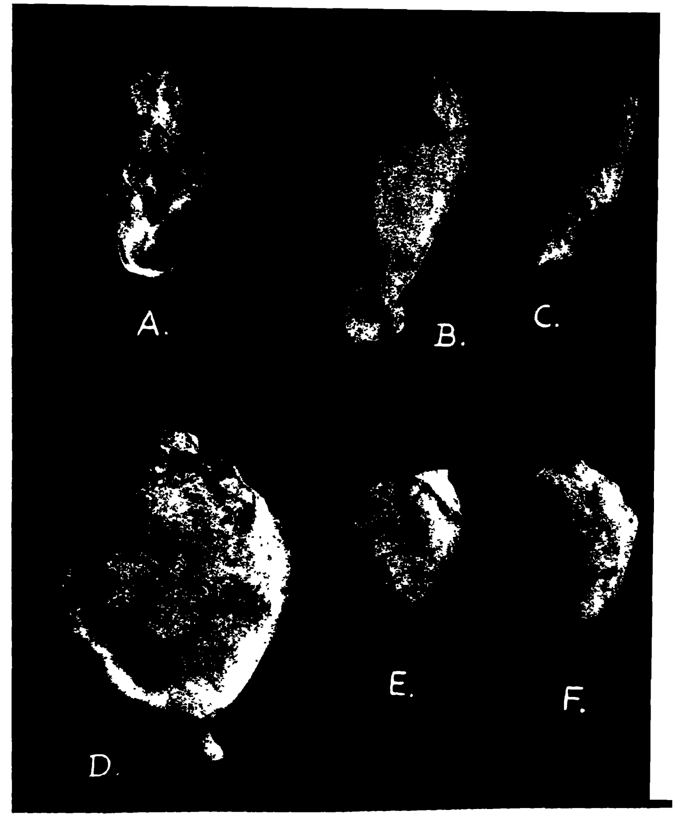


FIG. 2. *Normal and S. pullorum infected hearts. A, B, C, D—purulent pericarditis. E, F—normal hearts.*

TABLE II

RETESTS OF BIRDS SHOWING SUSPICIOUS OR FLUCTUATING REACTIONS TO THE AGGLUTINATION TEST

Number of bird	May	June	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Mar.	Apr.	May	June	July	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
145	$\frac{+}{25}$	$\frac{+}{25}$	$\frac{+}{25}$	$\frac{++}{25}$	$\frac{++}{10}$	$\frac{++}{25}$	$\frac{+}{10}$	$\frac{+++}{25}$	$\frac{+++}{25}$	$\frac{+}{25}$	–	–	$\frac{+}{25}$	–	$\frac{+}{10}$	–	–	–	–	–	–	–
159	$\frac{++}{25}$	$\frac{++}{10}$	$\frac{++}{50}$	$\frac{++}{25}$	$\frac{++}{25}$	$\frac{++}{50}$	$\frac{++}{25}$	$\frac{++}{25}$	$\frac{+++}{25}$	$\frac{++}{50}$	$\frac{+}{10}$	$\frac{+}{10}$	–	–	$\frac{+}{50}$	$\frac{++}{10}$	–	–	–	–	$\frac{++}{10}$	–
189	$\frac{++}{25}$	$\frac{+}{25}$	$\frac{+++}{25}$	$\frac{++}{25}$	$\frac{++}{25}$	$\frac{++?}{50}$	$\frac{+}{10}$	$\frac{+}{25}$	$\frac{+++}{25}$	$\frac{+}{50}$	0	$\frac{+}{25}$	–	–	–	$\frac{++}{50}$	–	–	–	–	–	–
191	$\frac{++}{50}$	$\frac{++}{25}$	$\frac{++?}{50}$	$\frac{++}{25}$	$\frac{+}{25}$	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
206	$\frac{++}{25}$	$\frac{+++}{25}$	$\frac{+++}{25}$	$\frac{++}{50}$	$\frac{++}{50}$	$\frac{+++}{50}$	$\frac{+++}{25}$	$\frac{++}{50}$	+++	$\frac{++}{25}$	0	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
255	$\frac{+++}{25}$	$\frac{+++}{10}$	$\frac{+++}{25}$	$\frac{++}{25}$	$\frac{+++}{25}$	–	–	–	$\frac{+++}{25}$	$\frac{+}{25}$	–	–	–	–	$\frac{+++}{10}$	$\frac{++}{10}$	–	–	–	–	–	$\frac{+++}{10}$
258	–	–	$\frac{+++}{25}$	$\frac{+}{25}$	–	$\frac{+}{25}$	–	–	–	$\frac{+}{25}$	$\frac{+}{10}$	$\frac{+}{25}$	$\frac{+}{10}$	$\frac{++}{10}$	$\frac{++}{10}$	$\frac{+}{10}$	$\frac{+}{25}$	$\frac{++}{10}$	$\frac{+}{10}$	$\frac{++}{10}$	–	–
297	+++	+++	+++	+++	+++	+++	$\frac{+++}{50}$	$\frac{+++}{50}$	$\frac{+++}{50}$	$\frac{++}{50}$	+++	$\frac{++}{50}$	$\frac{++}{50}$	$\frac{++}{50}$	$\frac{++}{100}$	$\frac{++}{50}$	$\frac{++}{50}$	$\frac{+++}{25}$	$\frac{++}{25}$	$\frac{++}{25}$	$\frac{+++}{10}$	$\frac{+}{25}$
173	–	–	–	$\frac{++}{100}$	$\frac{++}{50}$	$\frac{+}{25}$	$\frac{++}{25}$	–	–	–	–	Dead										
267	$\frac{++?}{50}$	$\frac{++}{25}$	$\frac{+++}{25}$	$\frac{+++}{50}$	+++	+++	+++	$\frac{++}{50}$	$\frac{+++}{25}$	$\frac{++}{50}$	$\frac{++}{100}$	$\frac{++}{100}$	Dead									

NOTE:—*Numerals indicate highest dilution at which reaction took place; where no numerals appear under a +++ sign, a positive reaction in a dilution of $\frac{1}{100}$ or $\frac{1}{100}$ and $\frac{1}{200}$ is indicated.*

– = *Negative reaction;* + = *Negative reaction;* ++ ? = *Suspicious reaction;* ++ = *Positive reaction;* +++ = *Positive reaction;* 0 = *Not tested.*

The evidence of progressive infection in this group of birds confirms the earlier work of Rettger *et al* (26), that pullorum disease spreads amongst mature fowls. In view of Kerr's (22) findings that carrier birds liberate *S. pullorum* in the faeces, and the recent work of Edwards and Hull (11), Brunett (6), Kernkamp (21), and Warrack and Dalling (32), that pullorum disease spreads amongst mature fowls, it is safe to conclude that the 12 originally non-reactors became actually infected in the course of the experiment.

Post-mortem examination of the same 12 birds revealed typical lesions of pullorum disease in all, while two were internal layers; *S. pullorum* was isolated from 10. In one case *S. pullorum* could not be isolated, while another bird was not examined bacteriologically, because of internal laying and advanced peritonitis.

Table II shows the degree of reaction in the highest dilution that occurred at each monthly retest. It will be seen that 5 out of the 10 birds never showed a distinct positive reaction in a 1:50 dilution. In routine testing these five birds would ordinarily have been considered negative, with the exception of one bird (No. 159), which in several retests gave a ++ reaction in a dilution of 1:50. On post-mortem examination the ovaries of these birds appeared normal, except that the ovary of one showed a flabby ovum, while the ovary of another showed one hemorrhagic and one flabby ovum. *S. pullorum* could not be isolated from any of these birds.

Three of the ten birds which showed, at the beginning of the experiment, a suspicious reaction in a dilution of 1:25 or 1:50, subsequently reacted consistently positive. *S. pullorum* was isolated from only two of the three birds, although typical lesions of pullorum disease were present in all of them.

One bird (No. 297) which gave a distinct +++ reaction in a dilution of 1:100 at the beginning of the experiment, showed a gradual decrease in the reaction and finally reacted negative. On post-mortem examination the ovary appeared normal; a culture which has not been identified as *S. pullorum* was isolated.

One bird (No. 173) showed inconsistent reactions to the agglutination test, having reacted respectively three times negative, twice positive, twice suspicious and four times negative. On bacteriological examination this bird was a non-reactor and the ovary appeared normal.

Considering the large percentage of reactors present in this group, the long contact period that existed between the reactors and non-reactors, and the consequent chances for infection, remarkably uniform results were secured in the retests.

Group 2

The 12 birds constituting this group were tested from 2 to 16 times. Four birds were tested 16 times, one 12, two 6, two 5, two 4 and one 3 times. These birds reacted consistently positive at all retests. A clear-cut reaction was obtained in the 1:100 dilution, and only a very few failed to react in the 1:200 dilution. There was also perfect agreement between the tests and the results of the post-mortem examination and cultural observations.

The flock from which these birds were secured was retested six weeks after removal of the reactors, at which time one more reactor was identified. Subsequent retest of the non-reactors of this flock and its pullet progeny showed that pullorum disease was eradicated from this flock, after the application of two tests.

Group 3

Thirteen White Leghorns in this group were under observation for 22 months and were tested 24 times in the author's laboratory and once in another laboratory. At the beginning of the experiment, *i.e.*, two and one-half months after the application of the routine test, a retest of the birds (first test of the experimental period) showed that one bird was negative to the test. This bird reacted negatively three consecutive times. Post-mortem and bacteriological examination confirmed the results of the repeated agglutination test.

A second bird, which showed a distinct +++ reaction in a dilution of 1:50 and 1:100 when tested by the previously mentioned laboratory, showed on retest a faint ++ reaction in a dilution of 1:50. In subsequent tests this bird showed considerable fluctuation in titre, although it never showed a distinct positive reaction in a dilution of 1:50 or 1:100. At time of post-mortem the bird was highly emaciated, and the ovary was completely dormant. *S. pullorum* was not isolated from this bird.

TABLE III

SUMMARY OF RETESTS OF 396 PULLETS TESTED THREE TIMES IN THE PULLET YEAR

	1st Test		2nd Test				3rd Test							
	Number birds tested	Number reactors	Number birds tested	Number reactors	Increase in reactors	Decrease in reactors	Number birds tested	Number reactors	Increase in reactors	Decrease in reactors	Total number reactors	Negative birds died	Positive birds died	Total birds died
S.C. W. Leghorns	114	9	107	8	1	3	96	4	0	0	9	12	3	15
R. I. Reds	105	13	96	13	3	1	86	9	4	0	20	11	8	19
B. P. Rocks	91	5	87	5	0	0	84	5	0	0	5	7	0	7
W. Wyandottes	51	13	44	11	1	1	38	14	6	0	20	8	5	13
Black Orpingtons	21	8	21	10	2	0	18	9	2	0	12	0	3	3
Light Sussex	14	2	14	2	0	0	13	2	1	0	4	0	1	1
Total	396	50	369	49	7	5	335	43	13	0	70	38	20	58

The remaining birds were tested as follows:— two, 24 times; two, 21 times; one, 15 times; one, 14 times; two, 10 times; one, 3 times; one, twice; and one, once. Each bird reacted consistently positive to each of the tests. The results of the post-mortem, bacteriological and serological tests were in perfect agreement.

Except for one bird, which had been originally recorded as a suspicious reactor, but reacted positive to the third test, pullorum disease would have

been eradicated from this flock, after two routine tests, conducted at six-month intervals. The pullets from this flock were not, however, free from pullorum disease. Unfortunately, the efforts to eradicate the disease failed, due to the known introduction of infected breeders early in the breeding season.

Group 4.

Pullets (396), of six breeds, were tested three times:— (1) at the commencement of the laying year; (2) in the middle of the laying year; and (3) at the end of the laying year. The birds of this group were not subjected to a post-mortem examination. The age of the non-reactors and reactors at the first test varied from 186 to 253 days. The average egg production of the whole flock was 21%, at the time of the first test, and only 15 out of the 50 reactors had laid.

A summary of the first, second and third tests is given in Table III. It will be seen that out of 396 pullets tested the first time, 50 pullets reacted positively to the agglutination test. Between the first and second tests, 27 birds died, of which 7 were reactors and 20 non-reactors. Of the 369 birds tested the second time, 49 pullets reacted positively. Seven of these 49 birds had reacted negatively to the first test. Five birds that had reacted positively to the first test failed to react to the second test. Between the second and third tests 34 birds died; of these 13 were reactors and 21 non-reactors, so that 335 birds were available for a third test. None of the birds that reacted positively to the second test reacted negatively to the third test. However, the five birds that reacted positively to the first test and reacted negatively to the second test, again reacted negatively to the third test. With the exception of these five birds the results of retesting reactors were consistent.

The apparent spread of infection in the non-reactors may have been due to direct contact with infected birds, contaminated droppings, feed and litter, or water, since no male birds were kept in this group. There is, of course, the possibility that some of the birds that reacted negatively to the first test but positively to the second test, had actually been infected with *S. pullorum* organisms prior to the first test, but had not developed sufficient agglutinins to react at that time. This possibility is however very remote in the case of the birds that reacted positively to the third test, but negatively to the second and first tests. It appears more probable that the close contact that existed between the non-reactors and reactors during the experiment was responsible for the development of infection and subsequent appearance of agglutinins in birds that reacted positively either to the second or third test. In this connection it should be noted that Hinshaw (16, 17) has observed that, even though reactors are removed from pullet flocks after each test, *infected* birds are subsequently detected. Presumably these birds were infected, but did not produce sufficient agglutinins to give a positive reaction. The time interval required to develop agglutinins would, on the basis of Hinshaw's results, be so short that at least the 13 birds that were negative on the second test but positive on the third, must have become infected subsequent to the first test.

At the end of the laying year all the reactors were removed. A fourth test

was conducted on the non-reactors kept for breeding purposes. No reactors were found, and the progeny from this group of birds was free from pullorum disease.

TABLE IV

REPRESENTATIVE TESTS OF MALE BIRDS

Male No.	1584						257						1446					
Dilution	R*	10	25	50	100	200	R	10	25	50	100	200	R	10	25	50	100	200
April	–	++	+	–	–	–	–	++	++	+	–	–	–	++	++	–	–	–
May	–	+++	+	–	–	–	+	+	+	++	+	–	–	+++	+++	++ ?	–	–
June	–	+++	+++	+	–	–	+	++	++	++	–	0	0	+++	+	–	–	0
June	–	+++	+++	++	–	–	++	+++	+++	+++	+	–	0	+++	++	–	–	–
July	–	+++	++	–	–	–	+++?	++	+++	–	–	0	–	++ ?	+	–	–	–
August	–	++	++	–	–	–	–	+++	++	+	–	–	–	0	0	–	–	–

Male No.	1312						1357						1					
Dilution	R*	10	25	50	100	200	R	10	25	50	100	200	R	10	25	50	100	200
April	+++	+++	+++	+++	++	–	+++	+++	+++	++	++	+	+++	+++	+++	+++	+++	–
May	+++	+++	+++	+++	++ ?	–	+++	+++	+++	++	++	+	+++	+++	+++	+++	+++	–
June	+++	+++	+++	+++	+	+	0	0	+++	+++	–	–	+++	+++	+++	–	–	–
June	+++	+++	+++	+++	+++	+	+++	+++	+++	+++	+++	+++	++	+++	+++	+	–	–
July	+++	+++	+++	+++	++	+	+++	0	++	++	++	+	+++	+++	+++	–	–	–
August	+++	+++	+++	+++	+++	+	+++	++	+++	++	+	+	+++	+++	+++	–	–	–

**R = Rapid method.* 0 = *Not tested.*

Group 5

Some of the male birds considered in this group were mated with the females of Groups 1, 2 and 3, while others were kept in a separate pen for retests only. Altogether 18 males were tested for various periods of time. Four of these were consistently negative even though kept in contact with reactors from seven to twenty months. On post-mortem examination these birds appeared perfectly normal. *S. pullorum* could not be isolated from the testes or pericardial sac. The remaining 14 males were considered as either suspicious reactors or reactors at the time the experiment was started. Ten of these reacted consistently positive, and were tested from 4 to 14 times, while the 4 suspicious males were tested 8, 12, 12 and 13 times respectively.

The results of some of the individual retests are presented in Table IV. Limitation of space does not permit a complete presentation of the retests of all the birds. However, the retests shown are characteristic of the group as a whole. It will be seen that there was considerable fluctuation in the reactions of the individual males from test to test. This is particularly true of males

whose sera agglutinated *S. pullorum* antigen in low dilutions only. Doyle's (10) observation that "the degree of reaction to the agglutination test in cock birds is less marked than in hens" is confirmed by the results secured in this investigation. In view of the difficulty of demonstrating lesions of pullorum disease or isolating *S. pullorum* from male birds, the significance of low agglutination titres is doubtful. The apparent discrepancies between the tube and rapid agglutination tests, in the reactions of certain male birds, further accentuate the difficulties of interpreting the reactions of male birds. In some cases

TABLE V

SUMMARY OF SEROLOGICAL, GROSS AND BACTERIOLOGICAL FINDINGS OF GROUPS 1, 2, 3 AND 5

Group	Number of birds		Pullorum lesions			*S. pullorum*		*N/E*
			P	*N*	*N/E*	*P*	*N*	
1	Negative	34		32	2		32	
	Positive	42	40	1	1	39	2	1
	N/P	12	12			10	1	1
	Suspicious	10	3	7		2	8	
2	Positive	12	12			12		
3	Negative	1		1			1	
	Suspicious	1		1			1	
	Positive	11	11			11		
5	Negative	4		4			4	
	Positive	10	3	7		5	5	
	Suspicious	4		4			4	

P = positive; N = negative; N/E = not examined.

this difficulty did not occur. Thus those males that showed a distinct +++ reaction in a dilution of 1:100 or 1:200 as a rule reacted consistently positive in the dilution considered diagnostic in these studies, *i.e.*, 1:50. It should be noted that in spite of variations in diagnoses from test to test, male birds that have once reacted positively to the agglutination test seldom show a complete loss of agglutinins.

Macroscopic lesions were found in three out of the ten reacting males and *S. pullorum* isolated from five. The males that gave a suspicious reaction appeared to be normal, and *S. pullorum* could not be isolated from either the pericardial sac or the testes.

Summary

The data presented in this paper are briefly summarized in Table V, which shows the results of serological, gross and bacteriological findings of birds in Groups 1, 2, 3 and 5. The results of Group 4 have already been shown in Table III. On this group of birds, no post-mortem examinations were conducted.

It will be seen from Tables III and V that a high degree of consistency was secured in retest of the five groups of birds obtained from various sources, and tested from two to twenty-two times. The results of the agglutination tests

were, except in a few cases, confirmed by the macroscopic appearance of the ovary and by bacteriological examination.

Acknowledgments

The author wishes to express his great appreciation to Miss Freda Handford, Miss Betty Guernsey, and Miss Vivienne Hudson, for assistance received in conducting the agglutination tests. To Miss Hudson particular credit is due for the technical help received in post-mortem examinations and cultural work.

References

1. Beach, B. A., Halpin, J. G. and Lampman, C. E. J. Am. Vet. Med. Asscn. LXX, n.s. 23: 605-611. 1927.
2. Beach, B. A., Holmes, C. and Strange, C. R. J. Am. Vet. Med. Asscn. LXXVI, n.s. 29: 557-561. 1930.
3. Beach, J. R. Hilgardia, 2: 529-544. 1927.
4. Biely, J. Sci. Agr. 9: 413-422. 1929.
5. Biely, J., Sawyer, C. E., Hamilton, C. M., Johnson, W. T. and Dickinson, E. M. J. Am. Vet. Med. Asscn. LXXIX, n.s. 32: 19-36. 1931.
6. Brunett, E. L. J. Am. Vet. Med. Asscn. LXXVI, n.s. 29: 667-669. 1930.
7. Bushnell, L. D. and Brandly, C. A. J. Am. Vet. Med. Asscn. LXXIV, n.s. 27: 444-453. 1929.
8. Dearstyne, R. S., Kaupp, B. F. and Wilfong, H. S. N. Carolina Agr. Exptl. Sta. Bull. 36: 3-53. 1929.
9. Doyle, T. M. J. Comp. Path. Therap. 38: 266-282. 1925.
10. Doyle, T. M. The Harper Adams Utility Poultry Journal, 12: 421-423. 1927.
11. Edwards, P. R. and Hull, F. E. J. Am. Vet. Med. Asscn. LXXV, n.s. 28: 333-336. 1929.
12. Edwards, P. R. and Hull, F. E. J. Am. Vet. Med. Asscn. LXXV, n.s. 28: 765-768. 1929.
13. Ericksen, S. Missouri State Poultry Asscn. Year Book, 31-33. 1923.
14. Fitch, B. S. and Lubbehusen, R. E. Proc. Third World's Poultry Congress, Ottawa, Canada, 356-360. 1927.
15. Gwatkin, R. Report, Ont. Vet. College, 44-64. 1925.
16. Hinshaw, W. R., Sanders, E. F. and Dunlap, G. L. Mass. Agr. Exptl. Sta. Control Ser. Bull. 43. 1928.
17. Hinshaw, W. R., Sanders, E. F. and Dunlap, G. L. Mass. Agr. Exptl. Sta. Control Ser. Bull. 48. 1929.
18. Jones, F. S. J. Med. Research, XXVII, n.s. 22: 481-495. 1913.
19. Kaupp, B. F. and Dearstyne, R. S. N. Carolina Agr. Exptl. Sta. Bull. 235: 3-16. 1928.
20. Kernkamp, H. C. H. Cornell Vet. 19: 357-367. 1929.
21. Kernkamp, H. C. H. J. Am. Vet. Med. Asscn. LXXVII, n.s. 30: 280-293. 1930.
22. Kerr, W. R. J. Comp. Path. Therap. 43: 77-85. 1930.
23. Lerche, Tierarztlichen Rundschau. 36: 332-335. 1930.
24. Lerche, Tierarztlichen Rundschau. 36: 346-350. 1930.
25. Newsom, I. E., Cross, F. and Ufford, O. C. J. Am. Vet. Med. Asscn. LXXII, n.s. 25: 611-617. 1928.
26. Rettger, L. F. and Stoneburn, F. H. Conn. Agr. Exptl. Sta. Storrs. Bull. 68. 1911.
27. Rettger, L. F., McAlpine, J. G. and Warner, D. E. J. Am. Vet. Med. Asscn. LXXVII, n.s. 30: 47-57. 1930.
28. Rice, J. P. Nat. Poultry J., v. 216. 1924.
29. Runnells, R. A. Va. Agr. Exptl. Sta. Bull. 265: 3-27. 1929.
30. Sawyer, C. E. and Hamilton, C. M. West. Wash. Exptl. Sta. Bull. 17-W. n.s. 1-19. 1930.

31. Tittsler, R. P., Heywang, B. W. and Charles, T. B. Penn. Agr. Exptl. Sta. Bull. 235: 3-16. 1928.
32. Warrack, G. H. and Dalling, T. Vet. J. 87: 24-27. 1931.
33. Wisconsin Agr. Exptl. Sta. Bull. 396. Ann. Rep. of the Director, 54-57. 1927.
34. Wisconsin Agr. Exptl. Sta. Bull. 405. Ann. Rep. of the Director, 31-32. 1929.
35. Wisconsin Agr. Exptl. Sta. Bull. 410. Ann. Rep. of the Director, 69-70. 1930.

INDEX TO VOLUME V

Authors

INDEX TO VOLUME V

Subjects

Date of Issue	Date of Issue	Date of Issue
9.4.58		